Effects of Reduced Cigarette Smoking on the Uptake of a Tobacco-Specific Lung Carcinogen

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Background: Limited data are available on carcinogen uptake in smokers who reduce their smoking. To determine whether reducing the number of cigarettes smoked per day would lead to a corresponding reduction in carcinogen uptake, we measured levels of metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butaneone (NNK) in the urine of smokers who reduced their smoking for up to 26 weeks. Methods: We recruited 153 smokers, of whom 151 were randomly assigned to a reduction group or a waitlist group. In the reduction group of 102 smokers, we measured the metabolites 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc) at two baseline times and at weeks 4, 6, 8, 12, and 26 after baseline. Smokers were then expected to reduce their number of cigarettes per day by 25% in weeks 0–2, 50% in weeks 2–4, and 75% in weeks 4–6 and to maintain the reduced level through week 26. In the waitlist group of 49 smokers, four baseline measurements over 7 weeks were made to assess the longitudinal stability of the metabolite measurements, and then the smokers began the reduction program. All statistical tests were two-sided. Results: For waitlist and reduction groups results were comparable. Statistically significant reductions in the lung carcinogen metabolites were observed at most intervals as smokers reduced the number of cigarettes smoked each day. However, the observed decreases were generally modest, always proportionally less than the reductions in cigarettes smoked per day, and sometimes transient. For example, among the 65 individuals in the reduction group who reduced cigarettes per day by 40% or more during weeks 4–12 after baseline, mean decreases in cigarettes per day were 53% (week 4), 74% (week 6), 75% (week 8), and 74% (week 12); whereas the corresponding mean reductions in NNAL plus NNAL-Gluc were 29%, 33%, 37%, and 29%. (P<.001 for all NNAL plus NNAL-Gluc values) Conclusions: Statistically significant reductions in levels of urinary metabolites of a tobacco-specific lung carcinogen were achieved by reduction in smoking, but for most smokers, reductions were modest and transient. [J Natl Cancer Inst 2004;96:107–15]
Subjects and Methods

Overview of Study Design

The study was approved by the University of Minnesota Research Subjects’ Protection Programs Institutional Review Board Human Subjects Committee. We recruited 153 smokers and randomly assigned 151 of them to a waitlist group or a reduction group. In the waitlist group, four measurements were made over a 7-week baseline period during ad libitum smoking. These four measurements would define biomarker longitudinal stability under baseline conditions, as well as providing a firm basis for comparison to biomarker levels during reduction. After the baseline period, the subjects in the waitlist group were enrolled into the reduction group. In the reduction group, two baseline values, obtained 1 week apart, were compared with the data obtained during reduction. For those individuals originally in the waitlist group, the first two baseline values that corresponded in time to the two baseline values from the subjects randomly assigned to the reduction group were used. To determine the effects of cigarette reduction on the biomarkers, smokers were asked to reduce their smoking optimally by 75%. Reduction was achieved through the use of behavioral treatment and medicinal nicotine. Biomarkers were measured at weeks 4, 6, 8, 12, and 26 after baseline. The study design is outlined in Fig. 1.

Details of Study Design

Cigarette smokers, aged 18–70 years, and interested in substantially reducing cigarette use but not quitting within the next 30 days, were recruited with advertisements on radio or in metropolitan and campus newspapers. Interested people telephoned our research clinic and were informed of the study goals and procedures. They were screened to determine whether they met specific inclusion criteria. These criteria included the following: 1) smoking 15–45 cigarettes per day for the past year; 2) in apparently good physical health with no unstable medical condition; 3) no contraindications for nicotine replacement use such as active ulcers, recent heart attack, heart disease or irregular heartbeat, high blood pressure not controlled by medication or medication use that might affect tobacco use or be affected by reduction of tobacco use; 4) in good mental health, e.g., not taking psychotropic medications or experiencing psychiatric diagnosis, including substance abuse, as determined by the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (18) criteria, within the past 6 months; 5) not using other tobacco or nicotine products; and 6) for female subjects, not pregnant or nursing.

Subjects meeting these criteria were asked to come to the research clinic for an orientation visit to provide informed consent and to be scheduled to return for a more thorough screening. During the orientation visit, subjects were also required to complete a tobacco use questionnaire and medical history form, which was reviewed by a physician.

Subjects who met inclusion criteria were asked to monitor their use of cigarettes and other tobacco products on a daily basis for 2 weeks to assess baseline tobacco use. Subjects were told that it was important to smoke cigarettes at their normal levels during this time. They returned the next week for the baseline visit (week -1, Fig. 1) and were assigned randomly to the reduction group or the waitlist group. We randomly assigned subjects to the waitlist group to define biomarker longitudinal stability at baseline and to verify results in the reduction group. Baseline measures were again taken at the subsequent visit (week 0). Those who were assigned to the waitlist group continued to smoke as usual for another 6 weeks, and those assigned to the reduction group began the reduction program.

Subjects assigned to the waitlist group were the basis for an analysis that examined the longitudinal stability of the biomarkers. This group was required to maintain and monitor smoking for 8 weeks. The first 2 weeks coincided with the baseline for the reduction group and the next 6 weeks coincided with the reduction phase. Subjects were assessed on all dependent measures during the first two baseline visits and then at week 4 and 6 after the onset of “treatment” in the reduction group. Subjects then entered the 26-week treatment reduction phase after the 6-week visit.

Subjects assigned to the reduction group were expected to reduce the number of cigarettes smoked per day by 25% in the first 2 weeks, 50% in the subsequent 2 weeks, and 75% in the next 2 weeks. They were given 4-mg nicotine gum and instructed on methods for assisting in reduction of cigarette smoking. Subjects kept track in their diaries of times they chewed gum. At each visit, if they were within two cigarettes of their goals, they were advised to continue use of the gum unless they had trouble with adverse events associated with it. Subjects who had difficulty reaching the 50% reduction goals were offered additional nicotine replacement. If a subject was unable to approach the 50% goal (more than two cigarettes from 50% reduction goal), he or she was offered the option of using a 14-mg Nicoderm CQ patch (a gift from SmithKline Beecham, Philadelphia, PA) in conjunction with nicotine gum for 2 weeks until the 75% reduction period. At the end of the 50% reduction period, if a subject was unable to approach the reduction goal or expressed concern about further reduction to 75%, an option of using a 21-mg Nicoderm CQ patch in conjunction with nicotine gum was offered. In addition to the pharmacologic therapies, subjects met with a trained counselor during the clinic visits for brief individual sessions lasting no more than 10 minutes. During these sessions, a specific, structured format was followed.

After the 6-week reduction period, subjects who were unable to reduce their smoking or who increased their smoking were discontinued from nicotine replacements and were followed.
Subjects who demonstrated some reduction in smoking were advised to sustain or reduce their level of smoking reduction to 50% or more of their baseline level or to quit. They were given nicotine replacements for another 6 weeks, during which time they were advised to use nicotine products as needed to sustain reduction or to quit, but with the goal of gradually reducing their use of nicotine gum over the 6-week period. After 12 weeks of being supplied medicinal nicotine products, they purchased their own products, if necessary. If at any time during or after the 6-week treatment sessions the subject reported wanting to quit, a target quit date was established, self-help treatment manuals were dispensed, and brief counseling was provided. Follow-up sessions occurred at 8, 12, and 26 weeks after the initiation of reduction.

Urine samples were collected to assess NNAL, NNAL-Gluc, cotinine, and anatabine. Carbon monoxide was determined in expired air. Subjective measures included a tobacco daily diary in which subjects were asked to record date and time of each cigarette smoked and nicotine gum chewed, and the use of any other tobacco product. In addition, a tobacco use questionnaire was also administered at the clinic visits. It asked about current tobacco use status, number of 24-hour or longer attempts at quitting, and the duration of abstinence from smoking during such attempts. Compliance in attending sessions was maximized by paying subjects cash for each visit. The amount paid per visit was dependent on the procedures for that visit.

To biochemically verify the self-reports of cigarettes per day, urinary anatabine levels were measured in all subjects who reported having reduced cigarettes per day by 40% or more during weeks 4–12 after baseline, or by 40% or more by week 4 and 70% or more during weeks 6–12 after baseline. Subjects were included in the biochemically verified groups if their anatabine levels were reduced by 30% or more at weeks 8 and 12 compared with baseline. This verification test was only applied to subjects whose baseline level of anatabine was more than 3.5 ng/mL. In subjects whose urinary anatabine concentration was below this level, exhaled carbon monoxide was used as a biomarker, and they were included in the biochemically verified group if their carbon monoxide levels were reduced by 30% or more at weeks 8 and 12 compared with baseline levels.

Biomarker Measurements

Alveolar carbon monoxide was measured at each visit with the Bedfont Micro Smokerlyzer (Bedfont, Medford, NJ). Subjects were asked to hold their breath for 15 seconds and expire into the device.

First morning urine voids (80 mL) were collected in 4-ounce (118-mL) polyethylene specimen collection jars (Fisher Scientific, Pittsburgh, PA). Four 4.5-mL aliquots were transferred to 5-mL cryotubes (Corning, Acton, MA). One tube was reserved, and the other three were used for various biomarker analyses. The urine remaining in the cup was also saved. Each sample was given a unique bar code label. All containers were maintained at −20 °C until analysis. NNAL and NNAL-Gluc were quantified as described (15,19). Anatabine was determined by a modification of a published method that used 5-ethyl-nornicotine as the internal standard (20). Further details of the method will be described elsewhere. Cotinine and its glucuronide (total cotinine) were quantified as described (15).

Simulations

Previous studies demonstrated that NNAL and NNAL-Gluc disappear slowly from urine after smoking cessation (15). Therefore, simulations were performed to determine the extent to which this could affect expected levels of NNAL and NNAL-Gluc in urine after a reduction of smoking. Using the average pharmacokinetic parameters determined from the urinary excretion of NNAL-Gluc in subjects who had stopped smoking (15) and the number of cigarettes smoked per day, we ran simulations with STELLA II (version 3.0.5 for Macintosh, High Performance Systems, Lebanon, NH). The reported number of cigarettes smoked per day for each subject was used, and a separate simulation was run for each subject. The baseline cigarette intake was assumed to be a steady-state intake, and thus the urinary NNAL-Gluc at baseline was considered to be a steady-state level of excretion. The simulation was begun by assuming that, for the next 4 weeks, the cigarette intake per day was that reported at week 4 after baseline; for weeks 4–6, the cigarette intake used was that reported at week 6; for weeks 6–8, the cigarette intake used was that reported at week 8; for weeks 8–12, the cigarette intake used was that reported at week 12. The output from the simulation was the urinary excretion rate of NNAL-Gluc normalized for creatinine excretion from weeks 0 to 12 for each subject.

Statistical Analysis

Statistical analysis was primarily used to determine whether a reduction in the number of cigarettes per day would lead to a reduction in NNAL, NNAL-Gluc, or total NNAL (the sum of NNAL and NNAL-Gluc). Our overall goal was to enroll 150 subjects with 100 subjects completing the study. Statistically significant reductions in biomarkers for disease have been observed in sample sizes as small as 16–33 individuals (17,21). Because the study was designed with each subject decreasing his or her cigarettes per day over time, time effects were examined. A linear mixed model for repeated measures was used to examine these time effects, thus taking advantage of the longitudinal design and avoiding inflated type I errors (22). Different covariance structures were considered and the structure with the lowest Akaike’s information criterion value (23) was selected in each model. NNAL, NNAL-Gluc, and total NNAL were analyzed on the logarithmic scale to satisfy normality and constant variance assumptions. Confidence intervals for NNAL, NNAL-Gluc, and total NNAL were formed at each time point with allowance for multiple decisions. The confidence intervals were constructed on the logarithmic scale and then back-transformed to the original scale. Confidence intervals for percent reduction in NNAL, NNAL-Gluc, and total NNAL were constructed on the exponential scale and then back-transformed to the original scale.

Because the intent of the study was to determine whether a reduction in cigarette smoking leads to a corresponding reduction in biomarkers, statistical analyses were carried out on subgroups of smokers defined by their success in following the reduction protocols, as well as on the waitlist group separately. An intent-to-treat population (e.g., all subjects who completed 26 weeks) would not be the most appropriate one for this analysis. All statistical tests were two-sided.
RESULTS

In the waitlist group, we performed four baseline measurements of NNAL and NNAL-Gluc, and these individuals joined the reduction group for 26 weeks. In the reduction group, we measured levels of NNAL and NNAL-Gluc at two baseline intervals and then 4, 6, 8, 12, and 26 weeks after baseline (Fig. 1).

The number of subjects recruited into the study was 153, with 151 of them randomly assigned to intervention groups: 49 subjects to the waitlist group and 102 to the reduction group. Of the subjects in the waitlist group, three dropped out before the baseline measurement and 13 dropped out during the reduction phase of the study. Of the participants who were assigned to the reduction group, none dropped out before the baseline measurement, and 37 dropped out during treatment (by week 12). Ninety-two of all participants who completed treatment were followed for 6 months.

Of the individuals who completed treatment and provided samples for analysis, the mean age was 46.8 years (standard deviation [SD] = 10.6; range = 20–80 years), number of cigarettes per day smoked at baseline was 23.7 (SD = 5.8; range = 15–46), total cotinine level at baseline was 2834 ng/mL (SD = 804–15340 ng/mL), duration of cigarette use was 28.6 years (SD = 10.8; range = 3–52 years), Fagerstrom Tolerance Questionnaire score was 4.4 (SD = 1.5), and mean number of attempts to quit was 3.5 (SD = 5.6). When compared with those who dropped out of the study, individuals who completed treatment were 6.0 years older (P < .001) and smoked 4.1 years longer (P = .003). There was no difference between these two groups in number of cigarettes per day smoked at baseline, number of years smoked, cotinine level, or Fagerstrom Tolerance Questionnaire score.

Although the study design called for staged reductions in cigarettes per day, many participants could not follow this protocol. We, therefore, defined the following subgroups of smokers: 1) reduced their cigarettes per day by 40% or more during weeks 4–6 after baseline; 2) reduced their cigarettes per day by 40% or more during weeks 4–12 after baseline with and without biochemical verification; and 3) reduced their cigarettes per day by 40% or more by week 4 and then 70% or more during weeks 6–12 after baseline.

Levels of NNAL, NNAL-Gluc, and total NNAL (NNAL plus NNAL-Gluc) per milligram creatinine in the urine of smokers who reduced their self-reported cigarettes per day by 40% or more during weeks 4–6 after baseline are summarized in Table 1. Effects of reduction in cigarettes per day on levels of total NNAL are shown in Fig. 2A; reduction in NNAL and NNAL-Gluc separately were similar to those for total NNAL. Statistically significant reductions in NNAL, NNAL-Gluc, and total NNAL, compared with baseline, were observed at all time points except week 26. The reductions in total NNAL at weeks 4 and 6 were 29% and 30%, respectively. Some individuals in this group relapsed after 6 weeks and were not able to maintain 40% reduction in cigarettes per day. At week 8, 12% had relapsed; at week 12, 21% had relapsed; and at week 26, 36% had relapsed. Nevertheless, average percent reductions in cigarettes per day were 66%, 63%, and 40% at weeks 8, 12, and 26 after baseline, respectively. The corresponding reductions in total NNAL at these time points were 35%, 28%, and 15%.

We examined levels of NNAL, NNAL-Gluc, and total NNAL per milligram creatinine in the urine of smokers who reduced their self-reported cigarettes per day by 40% or more during weeks 4–12 after baseline (Table 1; Fig. 2B). Mean decreases in cigarettes per day were 53%, 74%, 75%, and 74% at weeks 4, 6, 8, and 12, respectively. Statistically significant reductions in NNAL, NNAL-Gluc, and total NNAL per milligram creatinine were observed at all time points. The reductions in total NNAL...
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<th>No.</th>
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<td><strong>Baseline</strong></td>
<td>23.7 (22.6 to 24.8)</td>
<td>2.07 (1.78 to 2.29)</td>
<td>0.46 (0.39 to 0.53)</td>
<td>1.95 (1.40 to 2.72)</td>
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<td><strong>Week 4</strong></td>
<td>11.1 (10.5 to 11.7)</td>
<td>1.42 (1.24 to 1.62)</td>
<td>0.31 (0.26 to 0.36)</td>
<td>0.94 (0.85 to 1.22)</td>
<td>2.29 (1.95 to 2.72)</td>
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<td><strong>Week 6</strong></td>
<td>6.50 (5.97 to 7.04)</td>
<td>1.39 (1.20 to 1.59)</td>
<td>0.29 (0.24 to 0.35)</td>
<td>0.82 (0.73 to 0.92)</td>
<td>2.23 (1.95 to 2.72)</td>
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<td><strong>Week 8</strong></td>
<td>7.92 (6.87 to 8.96)</td>
<td>1.24 (1.07 to 1.45)</td>
<td>0.27 (0.23 to 0.33)</td>
<td>0.78 (0.71 to 0.84)</td>
<td>2.06 (1.63 to 2.61)</td>
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<td><strong>Week 12</strong></td>
<td>8.84 (7.42 to 10.3)</td>
<td>1.56 (1.44 to 1.68)</td>
<td>0.27 (0.22 to 0.34)</td>
<td>0.82 (0.76 to 0.88)</td>
<td>3.12 (2.33 to 4.16)</td>
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<td><strong>Week 26</strong></td>
<td>14.41 (12.4 to 16.4)</td>
<td>1.58 (1.22 to 2.06)</td>
<td>0.38 (0.29 to 0.51)</td>
<td>1.10 (0.87 to 1.48)</td>
<td>3.79 (2.51 to 5.72)</td>
<td>0.22 (0.18 to 0.28)</td>
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*CPD = cigarettes per day; NNAL = 4-(methyltritosamino)-1-(3-pyridyl)-1-butanol; NNAL-Glu = NNAL glucuronides; total NNAL = NNAL + NNAL-Glu; CO = carbon monoxide; NA = not analyzed. Values are reported as follows: top = geometric mean, except for CPD, which is the arithmetic, and 95% confidence interval; bottom = Number and P value, where statistically significant. All statistical tests were two-tailed. *Statistically significant. †Verified by anatabine and carbon monoxide data.

**Table 1. Effects of reduction in cigarettes per day (CPD) on levels of total NNAL, NNAL-Glu, and anatabine in urine, and carbon monoxide (CO) in expired air**
were 29%, 33%, 37%, and 29% at weeks 4, 6, 8, and 12, respectively (Fig. 2B); reductions for NNAL and NNAL-Gluc separately were similar to those for total NNAL. At week 26, only 32 of the 65 people in this group maintained reduction in cigarettes per day at 40% or greater; the rest relapsed or quit. Analyses of NNAL and NNAL-Gluc were available for 21 of these subjects. Figure 3 compares the percentage of reductions in cigarettes per day and total NNAL for these individuals with the corresponding reductions in all individuals who reduced cigarettes per day by at least 40% at weeks 4–6 after baseline and all individuals who reduced cigarettes per day by at least 40% in weeks 4–12. Reduction in total NNAL was substantially less at week 26 than at the earlier time points; the small number of subjects precluded statistical analysis at week 26.

Next, we included only individuals for whom self-reported data for reduction in cigarettes per day (40% or more at weeks 4 through 12) were biochemically verified by anatabine or carbon monoxide measurements (Table 1; Fig. 2C). The results were quite similar to those above, although the reductions in total NNAL and NNAL-Gluc were not statistically significant at week 12.

We carried out further analysis of smokers who reduced their self-reported cigarette consumption by 40% at week 4 and 70% at weeks 6–12 after baseline (Table 1; Fig. 2D). Reductions in NNAL, NNAL-Gluc, and total NNAL, expressed per milligram of creatinine, were statistically significant at all time points. Reductions of NNAL and NNAL-Gluc separately were similar to that shown in Fig. 2D for total NNAL. Reductions in total NNAL were 27%, 41%, 51%, and 46% at weeks 4, 6, 8, and 12, respectively. We also attempted analyses in a subset of 17 participants in this group for whom biochemical validation was available. Although this group was too small for rigorous statistical analysis, the results were similar to those in Table 1 and Fig. 2. Reductions in total NNAL, expressed per milligram creatinine, were 23%, 48%, 57%, and 55% at weeks 4, 6, 8, and 12, respectively.

We obtained similar results when we expressed NNAL, NNAL-Gluc, and total NNAL levels per milliliter of urine. Some individuals stopped smoking during the study. When we excluded these individuals from the analyses, the results were similar to those described above. We also stratified subjects by number of cigarettes per day at baseline, above and below the mean of 23.7. We did not observe consistent statistically significant differences from the data in Table 1 and Fig. 2.

The distribution half-lives ($t_{1/2 \alpha}$) of NNAL and NNAL-Gluc were approximately 3–4 days in smokers who quit, whereas the corresponding values for the terminal half-lives ($t_{1/2 \beta}$) of these compounds were 40–45 days (15). Consequently, we carried out simulations to determine whether pharmacokinetics could be used to predict the reduction in urinary levels of NNAL and NNAL-Gluc from the reduction in cigarettes per day. We found that the simulations could predict the expected percent reduction in NNAL-Gluc. For example, in the group that reduced their cigarettes per day by 40% or more in weeks 4–6 after baseline, simulations predicted that the maximum reductions in NNAL-Gluc at weeks 4, 6, 8, and 12 would be 49%, 68%, 68%, and 74%, respectively. The corresponding values for reductions in cigarettes per day were 53%, 73%, 66%, and 63%. However, both the simulations of reductions in NNAL-Gluc and the reduction in cigarettes per day over-predicted the observed reduction in urinary NNAL-Gluc. From a pharmacokinetic point of view, it would appear that even though the number of cigarettes per day was reduced, the actual dose of NNK obtained from each cigarette was increased.

Results of the analyses of carbon monoxide and anatabine are also presented in Table 1. Percent reductions in carbon monoxide were always greater than those of anatabine or total NNAL, and often approached percent reductions in cigarettes per day. For example, in the group that reduced cigarettes per day 40% or more in weeks 4–12, the biochemically verified reduction in carbon monoxide was 71% at week 12 compared with the reduction in cigarettes per day of 77% (Table 1). Percent reductions in anatabine at week 12 were similar to those of total NNAL.

Results of the analyses of the waitlist group are presented in Fig. 4. Included are all individuals who came for all baseline visits and then entered the reduction phase. There was no statistically significant difference among levels of total NNAL in urine during the four baseline visits. Mean reductions in cigarettes per day of 41%, 65%, 64%, 60%, and 34% were observed at weeks 4, 6, 8, 12, and 26 after baseline, compared with reduction in cigarettes per day at 40% or greater in weeks 4–12.

![Fig. 3. Percent reductions in cigarettes per day (open bars) and total 4-(methylamino)-3-pyridyl)-1-butanol (total NNAL = NNAL plus NNAL glucuronides [NNAL-Gluc]) per milligram creatinine (total NNAL = NNAL plus NNAL glucuronides) (solid bars) in smokers in the reduction group who reduced their self-reported smoking by 40% or more, during weeks 4–6, 4–12, and 4–26 after baseline. NNAL data are the geometric means. Error bars are upper limits of 95% CIs.

![Fig. 4. Cigarettes per day (CPD) and total 4-(methylamino)-3-pyridyl)-1-butanol (total NNAL = NNAL plus NNAL glucuronides [NNAL-Gluc]) per milligram creatinine in the urine of smokers in the waitlist group. Subjects in this group had four baseline measurements (weeks -1, 0, 4, and 6) and then entered the reduction group (see Figs.1 and 2). Diamonds and solid lines = CPD; squares and dashed lines = total NNAL per milligram creatinine; bars = 95% confidence intervals. Horizontal bar tops are longer for CPD than for total NNAL. *Statistically significant reduction compared with baseline. All statistical tests were two-sided. P values (mixed effects model fitting) for reduction in total NNAL (at week) were as follows: P = .010 (4 weeks); P < .001 (6 weeks); P < .001 (8 weeks); and P = .001 (12 weeks).]
corresponding, statistically significant reductions in mean total NNAL of 24%, 38%, 39%, 38%, and 22%.

Although many of the reductions in levels of NNAL, NNAL-Gluc, and total NNAL in Table 1 and Figs. 2–4 were statistically significant, the magnitudes of the reductions were generally modest compared with reductions in cigarettes per day. Reductions in cigarettes per day of 55%–90% during weeks 4–12 after baseline resulted in reductions of only 27%–51% in total NNAL (Table 1). Even when smokers reduced their cigarettes per day from a mean of 24.7 at baseline to 2.60 at week 12, a reduction of 90%, the mean level of total NNAL was still reduced only 46% from baseline (Table 1). Apparently, in this group, about 4.3 times more NNK was being obtained per cigarette at week 12 than at baseline.

We further investigated the relationship between cigarettes per day and total NNAL. We found that, although fewer cigarettes per day did not imply lower total NNAL values, lower total NNAL values always implied fewer cigarettes per day. Two groups of subjects, all non-abstinent, were examined at week 6 after baseline: one group of 31 individuals whose total NNAL value was reduced by more than 50% and the other group of 90 individuals who reduced their total cigarettes per day by 50% or more. In the first group, 30 of the 31 individuals reduced their cigarettes per day by more than 50%, but in the second group, only 30 of the 90 individuals had total NNAL values reduced by more than 50%. In fact, 18 individuals in the second group increased their total NNAL levels. Similar results were found at other time points in the study. These results demonstrate that achieving a reduction in total NNAL of greater than 50% requires a substantial reduction in cigarettes per day but that, conversely, a substantial reduction in cigarettes per day is not sufficient to achieve a corresponding reduction in total NNAL.

**Discussion**

The results of this study demonstrate that statistically significant reductions in urinary levels of NNAL, NNAL-Gluc, and total NNAL, metabolites of the tobacco-specific lung carcinogen NNK, can be achieved by reduction in smoking. In aggregate, however, the observed reductions in these metabolites were generally modest, less than reductions in cigarettes per day, and sometimes transient. Reductions in total NNAL were best in the group in which all smokers reduced their cigarettes per day by at least 70% in weeks 6–12 (Fig. 2D), but the average maximum reduction in total NNAL did not exceed 51% (at week 8). Further, among smokers who reduced their cigarettes per day to five or less, reductions in total NNAL were for the most part less than 50%. In all groups, reductions in total NNAL were smaller at the last interval studied than at the previous interval, e.g., week 26 compared with week 12 (Figs. 2 and 3). These results suggest that smokers compensated for the reduced numbers of cigarettes per day by smoking differently, thereby altering NNK delivery. Collectively, the results of this study indicate that, for most people, reduction in smoking is of limited value for reducing exposure to NNK.

In the only previous study examining the relationship between reduction in cigarettes per day and urinary NNAL, Hurt et al. (17) investigated subjects who used a nicotine inhaler for 12 weeks with follow up at 24 weeks; 23 subjects smoked a mean of 41.9 cigarettes per day at baseline, a higher rate than in our study. Only 16 subjects completed the 12-week inhaler treatment, with a mean reduction to 18.2 cigarettes per day at week 12 and to 26.7 cigarettes per day at follow up (week 24). Expired carbon monoxide levels were not statistically significantly reduced at any measured time point. NNAL, NNAL-Gluc, and total NNAL were not statistically significantly reduced at week 8. However, total NNAL (by 25%) and NNAL-Gluc (by 24%), but not NNAL, were statistically significantly reduced at week 24. Overall, these results were less consistent than ours with respect to reductions in carbon monoxide and urinary NNK metabolites, probably because of the smaller sample size and lower reductions in cigarettes per day than in our study.

Benowitz et al. (16) studied 13 smokers who smoked an average of 37 cigarettes per day. They reduced to 15, 10, or 5 cigarettes per day for 3–4 days. Nicotine, carboxyhemoglobin, and urinary mutagenicity declined but proportionately less than the change in cigarette consumption. The intake of tobacco toxins per cigarette increased roughly threefold when smokers reduced from 37 to five cigarettes per day. These results are quite consistent with ours, although different end points were examined.

Fagerstrom and Hughes (24) recently reviewed studies in which nicotine replacement therapy was used to reduce cigarettes per day, similar to the approach we used. A reduction in carbon monoxide (28%–31%) was observed in these studies, although the reduction was generally somewhat less than the reduction in cigarettes per day (43%–50%). Our results for reduction in carbon monoxide are consistent with these results. The reductions in carbon monoxide that we observed were generally greater than reductions in total NNAL or anatabine. Expired carbon monoxide has a short half-life and is a reliable biomarker of recent smoking (25). The greater reductions in carbon monoxide than in NNAL or anatabine may be related to its short half-life and/or to changes in subjects’ cigarettes per day with the approaching time for the next clinic visit.

Consistently, we observed that reductions in NNAL, NNAL-Gluc, and total NNAL were less than the corresponding reductions in cigarettes per day. Moreover, reductions in these metabolites were less at the last time point examined in each subgroup than at the previous interval. The most likely explanation for our results is compensation, a change in smoking behavior to adjust for different smoke yields (26). As clearly demonstrated by Benowitz et al. (16), smokers who decreased from 37 to five cigarettes per day in a short-term study, statistically significantly increased delivery of nicotine per cigarette. Increased puff volume is the most likely mechanism for this compensation (26). Using smoking machines, Fischer et al. (27) demonstrated that the total volume drawn through a cigarette was the main factor influencing NNK delivery in mainstream smoke. For example, as total volume increased from 200 mL to 800 mL (achieved by increasing puff volume), increases in NNK delivery up to threefold were observed in six different types of cigarettes. Djordjevic et al. (28) measured smoking topography and observed that larger puffs, shorter intervals between puffs, and larger total smoke volumes than specified in the Federal Trade Commission machine cigarette smoking protocol resulted in a 1.7-fold increase in NNK delivery. These observations could partially explain our finding that, among smokers who reduced from 24.7 to 2.60 cigarettes per day, NNK delivery per cigarette apparently increased 4.3-fold. The observation of smaller reductions in NNK metabolites at the last interval in
each group suggests that smokers were adjusting to their reduced smoking by compensation, although undetected relapse to higher than reported cigarettes per day is another possible explanation. Overall, the data suggest that our smokers changed their smoking topography, probably by increasing smoke volume, to compensate for reduced smoking and consequently blunted reductions in NNK intake.

Although decreases in NNK metabolites were modest compared with reductions in cigarettes per day, the 70% reduction group composed of 30 smokers did achieve an approximate 50% aggregate reduction in total NNAL at weeks 8–12 (Fig. 2D). This result suggests that some individuals may benefit from this approach. We could not identify any specific features such as sex, age, or cigarettes per day at baseline that statistically significantly distinguished this subgroup. Further studies in this direction are required. Sustained reductions of 50% in uptake of potent carcinogens such as NNK could have public health significance, particularly when one considers the magnitude of lung cancer mortality worldwide and the huge impact of cigarette smoking on this disease.

Whether a substantial number of smokers can achieve or sustain reductions of greater than 70% is uncertain and even questionable. Our study was not designed to examine the efficacy of methods to reduce smoking but, rather, to determine whether a reduction in the number of cigarettes smoked per day led to comparable reductions in levels of carcinogen biomarkers. Therefore, we did not attempt to use optimal smoking-cessation methods. A more effective method for cigarette reduction, for example, would not have required such a rapid reduction to 75% of baseline in cigarettes per day, particularly among smokers who wanted to reduce their smoking but were not ready to quit. Nonetheless, the percent of smokers randomly assigned to treatment who were able to sustain an approximate 50% reduction in smoking in this study are not unlike the rates observed in other studies. In one study (29) examining active versus placebo nicotine inhaler, 26% were able to sustain a 50% cigarette reduction at 4 months. In another study (30), 35% of smokers assigned to nicotine replacement were able to reduce their smoking by 50% or greater at 6 months. These results are similar to the 27% who self-reported sustained cigarette reduction at 6 months in our study. It is possible that more aggressive treatment is necessary to achieve reductions of greater than 70%.

The results of this study clearly demonstrate that total NNAL is a better biomarker than cigarettes per day for assessing reduction in carcinogen uptake. Our data show that dramatic reductions in cigarettes per day did not necessarily lead to corresponding reductions in total NNAL. Conversely, when total NNAL was reduced by at least 50%, there was frequently greater than 50% reduction in cigarettes per day. These results demonstrate the need for the use of objective carcinogen biomarkers in studies examining potential methods of harm reduction. Total NNAL in urine has many advantages as a biomarker, including direct relevance to carcinogenicity, practicality in measurement, and tobacco specificity (14).

A strength of our study was the use of urinary anatabine as a biomarker of compliance with the protocol (20). Because our subjects used nicotine replacement products, we could not use nicotine metabolites as biomarkers of reduction in smoking. Anatabine is a minor tobacco alkaloid that is not present in high quantities in nicotine replacement products. We biochemically verified reports of decreased cigarettes per day by urinary anatabine measurements. When baseline anatabine levels were lower than 3.5 ng/mL, we used exhaled carbon monoxide for verification. We were able to verify self-report in 54% of our subjects who reported reductions in cigarettes per day of 40% or more in weeks 4–12. Results for reductions in total NNAL were quite similar in the self-report and verified groups (Fig. 2B and C), indicating that self-report was generally accurate in these groups. Biochemical verification is not foolproof, however, because anatabine levels could be affected by compensation. We were unable to determine whether reductions in anatabine that were less than reductions in cigarettes per day were due to compensation or inaccurate reporting.

In summary, our results demonstrate that statistically significant reductions in NNAL, NNAL-Gluc, and total NNAL can be achieved by reducing cigarettes smoked per day. However, the reductions were generally modest and sometimes transient. Less than 30 of our subjects were able to achieve an approximate 50% reduction of total NNAL in 12 weeks, and this reduction required an average reduction of 70% or more in cigarettes per day. The results indicate that some smokers may benefit from reduced smoking, but for most the effects are modest, probably due to compensation.

REFERENCES

NOTES

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