Utility and Relationships of Biomarkers of Smoking in African-American Light Smokers

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Abstract

Although expired carbon monoxide (CO) and plasma cotinine (COT) have been validated as biomarkers of self-reported cigarettes per day (CPD) in heavy smoking Caucasians, their utility in light smokers is unknown. Further, variability in CYP2A6, the enzyme that mediates formation of COT from nicotine and its metabolism to trans-3'-hydroxycotinine (3HC), may limit the usefulness of COT. We assessed whether CO and COT are correlated with CPD in African-American light smokers (≤10 CPD, n = 700), a population with known reduced CYP2A6 activity and slow COT metabolism. We also examined whether gender, age, body mass index, smoking mentholated cigarettes, or rate of CYP2A6 activity, by genotype and phenotype measures (3HC/COT), influence these relationships. At baseline, many participants (42%) exhaled CO of ≤10 ppm, the traditional cutoff for smoking, whereas few (3.1%) had COT below the cutoff of ≤14 ng/mL; thus, COT seems to be a better biomarker of smoking status in this population. CPD was weakly correlated with CO and COT (r = 0.32-0.39, P < 0.001), and those reporting fewer CPD had higher CO/cigarette and COT/cigarette, although the correlations coefficients between these variables were also weak (r = -0.33 and -0.08, P < 0.05). The correlation between CPD and CO was not greatly increased when analyzed by CYP2A6 activity, smoking mentholated cigarettes, or age, although it appeared stronger in females (r = 0.38 versus 0.21, P < 0.05) and obese individuals (r = 0.38 versus 0.24, P < 0.05). Together, these results suggest that CO and COT are weakly associated with self-reported cigarette consumption in African-American light smokers, and that these relationships are not substantially improved when variables previously reported to influence these biomarkers are considered. (Cancer Epidemiol Biomarkers Prev 2009;18(12):3426–34)

Introduction

Biomarkers of cigarette smoke exposure have been used to confirm self-reported smoking measurements. Two commonly used measures are cotinine (COT) and exhaled carbon monoxide (CO). COT, a metabolite of nicotine, is detected in a number of biological fluids (i.e., plasma, saliva, and urine) and is highly specific to nicotine exposure (1). However, COT is not specific to cigarette smoke as individuals exposed to alternative sources of tobacco or nicotine replacement therapy will also have detectable COT (1). The traditional cutoff value for differentiating smokers from nonsmokers is plasma or salivary COT levels of ≤14 ng/mL (1, 2). COT has a relatively long half-life (13-19 hours) and reflects exposure to tobacco within the past 3 to 4 days (1, 3). CO is a byproduct of the combustion of tobacco, and a cutoff value for CO of ≤10 ppm is traditionally used to distinguish smokers from nonsmokers (1, 4). However, CO is not specific to tobacco smoke exposure and contributions from environmental sources (such as vehicle exhaust) and endogenous formation of CO from heme catabolism can limit its use (4). CO has a short half-life of approximately 1 to 4 hours (4) and reflects more recent exposure to smoking; it is highly dependent on the time of the last cigarette.

Several prior studies have confirmed the utility of both COT and CO levels to verify self-reported cigarette consumption, with correlation coefficients ranging from 0.3 to 0.8 (4–7). However, these studies have been done primarily among Caucasian moderate to heavy smokers. It is unknown whether these biomarkers are representative of cigarette consumption among light smokers where smoking levels are lower and occur at irregular intervals. In particular, the short half-life of CO, and its lack of specificity, may make it difficult to differentiate light smoking from nonsmoking. Although the longer half-life of CO may make it a suitable marker among light smokers, it may be influenced by variable rates of its metabolism. COT is the main proximate metabolite of nicotine (8), and COT itself is further metabolized to trans-3'-hydroxycotinine (3HC; refs. 9, 10). The conversion of nicotine to COT, and COT to 3HC, are primarily mediated by the enzyme cytochrome P450 2A6 (CYP2A6; ref. 11). Large

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interindividual variability in CYP2A6 activity has been reported (12). The gene encoding CYP2A6 is highly polymorphic, with 38 numbered alleles identified thus far. To aid in population studies, the 3HC/COT ratio (which can be measured in plasma, saliva, or urine) has been validated as a phenotype indicator of CYP2A6 activity (10). COT levels are potentially influenced by a number of factors that alter CYP2A6 activity, including genetic variation, the presence of enzyme inducers or inhibitors, body mass index (BMI), age, and gender. For instance, African-Americans have higher COT plasma levels than Caucasian smokers even after controlling for number of cigarettes smoked (13). This can be partly attributed to their slower rates of COT metabolism (14); ~50% of African-Americans have decrease- or loss-of-function CYP2A6 genetic variants compared with ~20% of Caucasians (12, 15). Furthermore, a large proportion of African-Americans smoke mentholated cigarettes, and it has been suggested that the cooling sensation may result in deeper, longer inhalation and larger puff volumes (16). Some studies, but not all, have found higher CO and COT levels among mentholated cigarette users (16).

There is also evidence that menthol can inhibit CYP2A6 in vitro and mentholated cigarette smokers have slower rates of nicotine metabolism (17, 18). Individuals with higher BMI have been found to have lower COT levels (5, 19), and older age has been associated with higher levels of COT (20, 21). Females are known to have faster rates of nicotine and COT metabolism (22), with estrogen being an inducer of CYP2A6 (23).

In this study, we examined whether biomarkers derived from ad libitum smoking are associated with self-reported cigarette consumption in a population of African-American light smokers, a population with unique smoking characteristics. The need for validated biomarkers for this specific population are warranted as the prevalence of light smoking is particularly common among African-Americans, with up to 50% reporting ≤10 cigarettes per day compared with 18% to 20% of the general smoking population (24). We also examined whether variables previously known to affect biomarker levels, such as rate of CYP2A6 activity (by genotype and 3HC/COT phenotype measure), gender, smoking mentholated cigarettes, BMI, or age influenced these relationships. Although CYP2A6 genotype was not found to substantially influence the relationships between biomarkers and cigarette consumption in a previous study of Caucasian heavy smokers (25), the proportion of individuals with CYP2A6 genetic variants was low. It is possible that the high prevalence of CYP2A6 decrease- or loss-of-function genetic variants and slower rates of COT metabolism among African-Americans may play a greater role in this population.

Materials and Methods

Study Design. A detailed description of the study design and participant recruitment can be found elsewhere (15, 26). Briefly, participants (n = 755) were randomized into a double-blind, placebo-controlled smoking cessation study at a community health center in Kansas City, Missouri. Participants (≥18 y of age) self-identified as “African-American” or “Black,” smoked ≤10 CPD for at least 6 mo before enrollment, and smoked at least 25 of the past 30 d were recruited. The research protocol was approved by the University of Toronto Ethics Review Office and the University of Kansas Human Subjects Committee.

Baseline Assessment. Information regarding the participant’s demographic, smoking, and psychosocial characteristics have been described in detail elsewhere (27). Age, gender, and BMI were collected at randomization. Participants were asked about their smoking patterns, including the number of CPD, mentholated versus nonmentholated cigarette use, depth of inhalation, and number of years smoked. Participants were asked to estimate their cigarette consumption by the question: “During the past 7 days, on those days that you smoked, what was the average number of cigarettes smoked per day?” Although all of the participants recruited into the clinical trial reported smoking ≤10 CPD on the eligibility questionnaire, a small subset of participants reported consuming zero or >10 CPD during the past week during the randomization assessment (n = 55) and thus were excluded from analyses in the current study.

Biochemical Measures. A blood sample was collected at randomization to determine the levels of nicotine and its metabolites, and for genotyping purposes. Plasma levels of nicotine, COT, and 3HC were determined using the methods described elsewhere (10), although 3HC levels were only available for a subset of the participants (n = 602 of 700).Expired CO levels were measured by a handheld portable CO monitor (Bedfont Micro Smokerlyzer).

CYP2A6 Genotyping. CYP2A6 genotyping for this population was done using two-step allele-specific PCR assays as described previously (15). A subset of the total participants consented to have their blood sampled for genetic analyses, and genotyping data were available for 570 of the 700 participants. Participants were genotyped for CYP2A6*1B, *2, *4, *9, *12, *17, *20, *23, *24, *25, *26, *27, *28, and *35 (15). Individuals were categorized into groups based on their predicted effects of their CYP2A6 genotypes on rates of activity as previously described (15). Those with one copy of the decrease-of-function alleles (CYP2A6*9 and *12) were grouped as intermediate metabolizers (n = 70). Individuals with two copies of the decrease-of-function alleles, one or two copies of loss-of-function alleles (CYP2A6*2, *4, *17, *20, *23, *24, *25, *26, *27, and *35), or one decrease-of-function allele with one loss-of-function allele were grouped as slow metabolizers (n = 197). Normal metabolizers (n = 275) were individuals without these genetic variants. Those with CYP2A6*1B (n = 89) were also included in the normal metabolizer group as previously described (15). Individuals with CYP2A6*28 (n = 28) were excluded from analyses due to extreme range in 3HC/COT values, suggesting some may also have gain-of-function copy number variants, which is currently under investigation.

Statistical Analyses. Statistical analyses were done using SPSS statistical software, version 16.0. The data (CPD, CO, nicotine, COT, 3HC, 3HC/COT, BMI) were not normally distributed according to the Kolmogorov-Smirnov test and were log transformed when appropriate. Pearson’s correlation coefficient was used to examine the
relationships between log-transformed CPD, CO, and COT with CO/cigarette, COT/cigarette. Differences in log-transformed CPD, CO, or COT between gender, use of mentholated cigarettes, and BMI as categorized by those considered obese (BMI, ≥30) versus nonobese (BMI, <30), were tested using the t test for independent samples. Differences in CPD, CO, or COT between CYP2A6 genotype groups, 3HC/COT quartiles, and age categories were examined using univariate ANOVA with Bonferroni correction for post hoc analyses. Pearson’s correlations between log-transformed CPD, CO, or COT with age and log-transformed 3HC/COT and BMI as continuous variables were also examined. Differences between Pearson’s correlation coefficients were tested using the Fisher r-to-z transformation. A multiple linear regression model was used to examine the predictors of baseline CPD, CO, and COT. Variables included in the model were significant in univariate analyses (P < 0.10), and variables that were not normally distributed (CPD, CO, COT, BMI, 3HC/COT) were log-transformed in the analyses.

Results

Participant Demographics. A summary of the participant demographics, smoking history, and biochemical measures can be found in Table 1. The study sample was overrepresented by females (66.7%), and the majority smoked mentholated cigarettes (81.3%). A histogram of the CPD, expired CO, and plasma COT from baseline smoking is shown in Fig. 1A to C. Many participants (42%, n = 294) had expired CO values of ≤10 ppm, the traditional cutoff for differentiating between smokers from nonsmokers. In contrast, few individuals had plasma COT levels below the widely used cutoff value of 14 ng/mL (3.1%, n = 22; refs. 1, 3), or below the cutoff of 20 ng/mL used to verify smoking abstinence in this clinical trial (3.9%, n = 27; ref. 26).

Relationship Between Expired CO, Plasma COT, and CPD. CPD was significantly, albeit weakly, correlated with expired CO (Pearson’s r = 0.32, P < 0.001) and with plasma COT (Pearson’s r = 0.39, P < 0.001; Fig. 1D and E). Expired CO and plasma COT were also significantly correlated with each other (Pearson’s r = 0.60, P < 0.001; Fig. 1F). We examined the ratio of expired CO or plasma COT to self-reported values of cigarettes smoked as indicators of the extent of inhalation. CPD was poorly correlated with CO/ cigarette (Pearson’s r = −0.33, P < 0.001; Fig. 1G) and COT/cigarette (Pearson’s r = −0.08, P < 0.05; Fig. 1H).

Variables that Influence CPD, Expired CO, and Plasma COT. CYP2A6 slow metabolizers, as defined by genotype, had significantly higher plasma COT levels compared with normal metabolizers (P < 0.01), although CYP2A6 genotype was not associated with CPD or expired CO levels (Table 2; Fig. 2A-C). Similarly, individuals with 3HC/COT ratios in quartile 1, representing those with slowest rate of CYP2A6 activity, also had significantly higher plasma COT levels compared with those in quartile 4 (P < 0.001), although the 3HC/COT ratio was not significantly associated with either CPD or expired CO (Table 2; Fig. 2D-F). CYP2A6 slow metabolizers by genotype were also found to have significantly higher plasma nicotine (P < 0.001) and 3HC levels (P < 0.001), and those in the slowest 3HC/COT quartile had significantly higher plasma nicotine and 3HC levels compared with those with the fastest metabolic activity (P < 0.001).

No gender difference was found for CPD or expired CO, although females trended toward higher plasma CO levels (P = 0.09; Table 2). Those who smoked mentholated cigarettes trended toward reporting fewer CPD compared with those who did not (P = 0.05), although no difference was found for expired CO or plasma COT levels between mentholated and nonmentholated cigarette smokers. Obese individuals (BMI, ≥30) smoked fewer cigarettes (P < 0.05) and had lower levels of expired CO (P < 0.05) and COT (P < 0.001). BMI was not significantly correlated with CPD, but a negative correlation was found between BMI and expired CO (P < 0.01), and between BMI and plasma COT (P < 0.001). CPD, expired CO, and plasma COT were not significantly associated with age, either by correlation analyses or when examined categorically. We did not find significant differences in CPD, expired CO or plasma COT by self-reported inhalation patterns or the presence of other smokers in the home (Table 2).

Variables that Influence Relationships Between CPD and Expired CO with Nicotine and Its Metabolites. The correlation coefficients between CPD and expired CO with plasma nicotine and its metabolites were not greatly altered by CYP2A6 genotype or 3HC/COT quartiles (Table 3). Similarly, these relationships were generally not altered when analyzed separately by gender, mentholated cigarettes, BMI, or age. However, it is notable that the correlation between CPD and expired CO was stronger in females compared with males (Pearson’s r = 0.38 versus 0.21, respectively; P < 0.05) and in obese (BMI, ≥30) compared with nonobese individuals (Pearson’s r = 0.38 versus 0.24, respectively; P < 0.05). No difference was observed for the correlations between CPD and plasma COT was observed by gender or BMI.

In a multiple regression model including the predictors of CPD that were significant in univariate analyses (P < 0.10), plasma COT (β = 0.32) and expired CO (β = 0.12) remained significant (P < 0.01), whereas BMI was no
longer significant, and the trend of mentholated cigarettes use associating with fewer CPD remained (Table 4). Together, these predictors accounted for 17% of the variance in CPD.

Discussion

In this population of African-American light smokers, where approximately one-third consume ≤ 5 CPD, two commonly used biomarkers of cigarette smoke exposure, expired CO and plasma COT, were significantly correlated with self-reported CPD. However, the strength of the correlations were relatively weak (r = ~ 0.31-0.37), and in a multiple regression model, only ~17% of the variance in CPD was explained by plasma COT and expired CO. This is in contrast to heavy smoking Caucasian populations, where correlation coefficients from 0.3 to 0.8 have been reported (4-7). Self-reported number of CPD is a limited indicator of exposure as there is a nonlinear relationship between biomarkers and CPD, with a plateau observed at higher levels of consumption (>20-25 CPD; ref. 28). Heavy smokers reporting consumption at these levels seem to smoke each cigarette with less intensity (25, 28). Thus, self-reported measures of CPD may not be representative of exposure particularly at extremely high or low levels of smoking.

It would have been ideal to compare our findings with a matched group of Caucasian light smokers from a clinical trial (e.g., treatment seekers) to determine whether the weaker correlations between the biomarkers and self-reported cigarette consumption in this study were reflective of variables that were specific to African-Americans, or resulted from the narrow range in cigarettes consumed. However, established light smoking patterns among adults is less common among Caucasians, and the clinical trial from which participants in the current study was drawn is the only published one to date to have recruited specifically light smokers (≤10 CPD; ref. 29). To partially address this issue, we analyzed a subset of Caucasian smokers that reported ≤ 10 CPD in our previously published biomarker article (25). Despite the considerably smaller numbers in this subset analyses (n = 40), the correlation coefficients seemed higher in the Caucasian light smokers. Specifically, the Pearson’s correlation coefficient between CO with CPD (r = 0.37, P = 0.02) was similar, whereas the correlations between COT with CPD (r = 0.53, P < 0.001) and CO with COT (r = 0.74, P < 0.001) remained similar. Thus, it seems that CO may be a poorer biomarker of cigarette consumption in African-American light smokers compared with Caucasians, and as expected, CO seems poorly correlated with CPD in all light smokers. Furthermore, in a subset of heavy-smoking, treatment-seeking African-Americans, in which these variables were available, recruited for a clinical trial testing the efficacy of bupropion (n = 93; ref. 30), both CO and COT were poorly correlated with self-reported cigarette consumption (r = 0.20, P = 0.05

Figure 1. A to C. Histogram of self-reported CPD and the biomarkers expired CO and plasma COT for study participants (n = 700). Cutoff values of expired CO at ≤10 ppm, and plasma COT levels of ≤14 ng/mL have been traditionally used to differentiate smokers from nonsmokers. Correlations are weak but significant between expired CO with CPD (D), plasma COT with CPD (E), and plasma COT with CO (F). Correlations are also significant between CO/cigarette with CPD (G) and COT/cigarette with CPD (H). Each point represents an individual. r = Pearson’s correlation coefficient. Analyses were done on log-transformed variables (CPD, expired CO, plasma COT), although raw data are plotted.


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for CO with CPD, and \( r = 0.05, P = 0.62 \) for COT with CPD). Together, this suggests that CO is a poor correlate of cigarette consumption in light smokers in general, whereas COT is a poor correlate of cigarette consumption among African-American smokers.

Traditional cutoff levels of expired CO and plasma COT for differentiating between smokers and nonsmokers have previously been determined primarily in heavy smoking Caucasian populations (2, 31). Our results suggest that using expired CO of \( \leq 10 \) ppm to verify smoking status in light smokers may result in misclassification of smokers as nonsmokers, as \( \sim 40\% \) of our treatment-seeking sample of smokers had expired CO levels below this limit. In contrast, very few individuals (3.1\%) had plasma COT levels below the traditional cutoff of 14 ng/mL. This cutoff value was determined 20 years ago when there were high levels of secondhand smoke (2). Recently, it was suggested that the plasma COT cutoff should be further reduced to 3 ng/mL, with optimal cutoff revised to 6 ng/mL for African-Americans (32). This revised cutoff of 6 ng/mL would misclassify only 2.5\% of smokers as nonsmokers in this sample. Although further studies will be needed to precisely determine the optimal cutoff points for expired CO and plasma COT.

### Table 2. Variables that influences CPD, expired CO, or plasma COT levels

| Variable                              | CPD Mean  | CPD SD  | CPD n  | CPD P  | Expired CO (ppm) Mean  | Expired CO (ppm) SD  | Expired CO (ppm) n  | Expired CO (ppm) P  | Plasma COT (ng/mL) Mean  | Plasma COT (ng/mL) SD  | Plasma COT (ng/mL) n  | Plasma COT (ng/mL) P  |
|---------------------------------------|-----------|---------|--------|--------|------------------------|----------------------|---------------------|-----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| CYP2A6 genotype                       |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| NM                                    | 7.04      | 2.51    | 275    | 0.85   | 14.33                  | 9.24                 | 274                 | 0.13                  | 221.74                 | 146.31                 | 275                    | 0.002                  |
| IM                                    | 6.97      | 2.96    | 70     | 0.01   | 11.94                  | 6.90                 | 70                  | 0.05                  | 249.17                 | 149.06                 | 70                     | 0.002                  |
| SM                                    | 7.14      | 2.44    | 197    | 0.03   | 13.53                  | 9.22                 | 197                 | 0.12                  | 272.65*                | 165.17                 | 197                    |                        |
| 3HC/COT ratio                         |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Pearson’s r                           | 0.01      | 0.98    |        | 0.06   | 0.12                   |                       | 0.28                |                       |                       | 0.01                   | 0.01                   |                        |
| Categorical                           |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Fastest—quartile 4                    | 7.22      | 2.50    | 151    | 0.01   | 12.95                  | 9.35                 | 151                 | 0.85                  | 189.25                 | 127.20                 | 151                    | <0.001                  |
| Quartile 3                            | 7.28      | 2.48    | 150    | 0.67   | 14.45                  | 9.72                 | 150                 | 0.62                  | 255.45                 | 147.85                 | 150                    | 0.002                  |
| Quartile 2                            | 7.07      | 2.35    | 197    | 0.03   | 14.10                  | 8.52                 | 197                 | 0.12                  | 265.94                 | 146.11                 | 151                    |                        |
| Slowest—quartile 1                    | 7.22      | 2.44    | 197    | 0.03   | 12.89                  | 6.88                 | 197                 | 0.08                  | 278.62                 | 170.57                 | 150                    | 0.002                  |
| Gender                                |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Males                                 | 7.04      | 2.55    | 233    | 0.02   | 13.42                  | 8.19                 | 233                 | 0.63                  | 231.79                 | 150.28                 | 233                    | 0.09                   |
| Females                               | 7.20      | 2.58    | 467    | 0.04   | 13.88                  | 8.98                 | 466                 | 0.04                  | 249.60                 | 153.84                 | 466                    |                        |
| Menthol cigarettes                    |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Mean                                  | 7.07      | 2.46    | 569    | 0.05   | 13.49                  | 8.28                 | 569                 | 0.51                  | 242.93                 | 150.55                 | 568                    | 0.55                   |
| Yes                                   | 7.53      | 2.32    | 131    | 0.01   | 14.74                  | 10.42                | 131                 | 0.05                  | 246.84                 | 162.71                 | 131                    |                        |
| No                                    |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| BMI                                   |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Pearson’s r                           | −0.03     | 0.39    |        | −0.11  | 0.004                  | 0.003                | −0.19               |                       | −0.19                  | <0.001                 |                        |                        |
| Categorical                           |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Low (<30.0)                           | 7.35      | 2.41    | 381    | 0.02   | 14.35                  | 8.74                 | 380                 | 0.01                  | 268.19                 | 156.37                 | 380                    | <0.001                 |
| High (≥30.0)                          | 6.90      | 2.46    | 316    | 0.02   | 12.97                  | 8.70                 | 316                 | 0.01                  | 213.57                 | 142.65                 | 316                    |                        |
| Age                                   |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Pearson’s r                           | 0.05      | 0.21    |        | −0.001 | 0.04                   | 0.34                 | 0.04                |                       | 0.04                   | 0.34                   |                        |                        |
| Categorical                           |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| 19-29                                 | 6.32      | 2.27    | 60     | 0.18   | 11.72                  | 5.68                 | 60                  | 0.95                  | 226.14                 | 147.88                 | 60                     | 0.42                   |
| 30-39                                 | 7.20      | 2.36    | 148    | 0.01   | 14.14                  | 9.80                 | 148                 | 0.01                  | 241.29                 | 149.26                 | 148                    |                        |
| 40-49                                 | 7.16      | 2.48    | 280    | 0.01   | 13.75                  | 8.54                 | 280                 | 0.01                  | 239.85                 | 158.70                 | 279                    |                        |
| 50-59                                 | 7.41      | 2.45    | 158    | 0.01   | 13.77                  | 8.30                 | 157                 | 0.01                  | 255.99                 | 151.70                 | 158                    |                        |
| 60-77                                 | 7.04      | 2.51    | 52     | 0.01   | 14.29                  | 10.51                | 52                  | 0.01                  | 248.82                 | 141.69                 | 52                     |                        |
| Depth of inhalation                   |           |         |        |        |                        |                      |                     |                       |                        |                        |                        |                        |
| Mean                                  | 7.31      | 2.45    | 426    | 0.13   | 14.00                  | 9.08                 | 426                 | 0.01                  | 242.19                 | 156.54                 | 382                    | 0.50                   |
| No                                    | 7.24      | 2.48    | 427    | 0.01   | 13.84                  | 8.28                 | 425                 | 0.07                  | 256.01                 | 160.08                 | 426                    | 0.19                   |

*Denotes statistical significance in plasma COT levels in normal metabolizers vs slow metabolizers.

†Denotes statistical significance in plasma COT levels in quartile 4 (fastest activity) vs quartile 1 (slowest activity). No significant differences in plasma COT levels were found between intermediate metabolizers vs normal metabolizers or slow metabolizers, or between the other quartiles. Raw data are listed although statistical analyses were done on log-transformed data for CPD, expired CO and plasma COT.
among African-American light smokers, our study suggests plasma COT may be a better indicator of smoking status than expired CO.

The second objective of this study was to determine whether other variables (i.e., CYP2A6 activity, gender, age, BMI, smoking mentholated cigarettes) influence biomarkers levels (expired CO, plasma COT) or their relationships to self-reported CPD. Individuals with slow CYP2A6 activity, as indicated by genotype and 3HC/COT, had significantly higher plasma COT levels despite similar intake as represented by self-reported CPD and expired CO values. COT clearance rates were previously found to be reduced by ~35% in individuals with CYP2A6 genetic variants (33), and in this study, COT levels were ~20% to 30% higher in individuals with slow CYP2A6 activity. Despite its substantial effect on COT levels, however, CYP2A6 activity did not greatly alter the correlations between nicotine or its metabolites with self-reported CPD or expired CO levels, similar to what was observed in our previous study of Caucasian heavy smokers (25).

Individuals with higher BMI were found to have significantly lower plasma COT levels (r = −0.19), as well as lower nicotine (r = −0.16) and 3HC (r = −0.26) in this study. A negative correlation between BMI and COT levels has been previously reported (r = −0.16 to −0.36; refs. 5, 19). It is possible that differences in BMI may result in altered volumes of distribution for nicotine and its metabolites, thus resulting in altered plasma levels. The volume of distribution for nicotine and COT have been correlated with total body weight and lean body mass (r = 0.23-0.67), although no significant correlation was found between the volume of distribution with adipose mass (14). BMI has also been negatively associated with the 3HC/COT ratio (15, 34, 35), suggesting obesity and rate of CYP2A6 activity may be related, although this has not been examined explicitly. Although the lower plasma COT levels in individuals with higher BMI may also be interpreted as lower exposure to cigarette smoke, this is unlikely as BMI was not significantly associated with expired CO or CPD in multiple regression analyses. As such, it is yet unclear whether the relationship between BMI and COT represents altered rates of COT metabolism (altered CYP2A6 activity), or volume of distribution, or a combination of both. Interestingly, expired CO seemed to be a better measure of exposure in obese individuals (BMI, ≥30.0) as the correlation to CPD seemed stronger in these individuals.

It has been proposed that the cooling sensation associated with smoking mentholated cigarettes allows for increased smoke intake. Thus, this may contribute to the higher COT levels and disproportionately higher incidences of tobacco-related illnesses among African-Americans, who, because of the influence of marketing campaigns in the 1960s, predominantly smoke mentholated cigarettes (36). However, although some cross-sectional and experimental studies have found higher CO and COT levels among mentholated cigarette smokers, this has not been consistently replicated (16). Studies finding an effect were generally of small sample size, and included both heavy-smoking Caucasians and African-Americans. In this current study of African-American light smokers, mentholated cigarette users did not have significantly higher expired CO or plasma COT levels despite our large sample, with 131 nonmenthol smokers examined. Thus, cigarette mentholation did not seem to contribute to increased intensity of cigarette smoking or increased absorption of nicotine in our sample of African-American light smokers.

No differences in CPD, expired CO, or plasma COT were found by age, in contrast to previous findings where

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Relationship between CYP2A6 activity, CPD, expired CO, and plasma COT. Self-reported CPD and expired CO did not differ by CYP2A6 genotype (A and B) or 3HC/COT quartiles (D and E), whereas CYP2A6 slow metabolizers had significantly higher plasma COT levels compared with normal metabolizers (*, P < 0.01; C) and those in the slowest 3HC/COT quartile had significantly higher plasma COT levels compared with those in the fastest quartile (#, P < 0.001, F). NM, normal metabolizers; IM, intermediate metabolizers; SM, slow metabolizers. Analyses were done on log-transformed variables (CPD, expired CO, plasma COT), although raw data are plotted.
older individuals had higher COT levels (20, 21). In general, drug metabolism is thought to decrease by age (37), and although nicotine clearance rates are reduced in the elderly (age >65 y), this has been attributed to age-related changes in hepatic blood flow as no differences in CYP2A6 protein by age have been observed (38). The renal clearance of COT is also reduced in the elderly (age >65 y), this has been attributed to age-related changes in hepatic blood flow as no differences in CYP2A6 protein by age have been observed (37), and although nicotine clearance rates are reduced in the elderly (age >65 y), this has been attributed to age-related changes in hepatic blood flow as no differences in CYP2A6 protein by age have been observed (37). The renal clearance of COT is also reduced in the elderly, although pharmacokinetic parameters such as area-under-the-curve and elimination half-lives are weaker relationships between self-reported CPD and expired CO.

One limitation of our study is that this was a secondary analyses performed on participants that were originally recruited for a clinical trial on smoking cessation, and may not be representative of African-American light smokers in the general population. Thus, biochemical measures were collected randomly from ad libitum smoking and the time of the last cigarette may have been a significant source of variation, particularly for expired CO where the half-life is short. It is possible that this was a significant source of variation, particularly for expired CO where the half-life is short. It is possible that this was a significant source of variation, particularly for expired CO where the half-life is short. It is possible that this was a significant source of variation, particularly for expired CO where the half-life is short.

When analyzed separately by variables of interest

### Table 3. Correlations (r) between biomarkers and cigarette consumption by variables

<table>
<thead>
<tr>
<th>Total population (n = 700)</th>
<th>CYP2A6*1/*1 only (n = 275)</th>
<th>CYP2A6 variants only (n = 267)</th>
<th>3HC/COT* Quartiles 2-4 (n = 451)</th>
<th>Slowest quartile only (n = 151)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expired CO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (n = 233)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females (n = 467)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke nonmentholated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cigarettes (n = 131)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke mentholated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cigarettes (n = 569)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Values listed are Pearson’s correlation coefficients calculated on log-transformed variables (CPD, expired CO, plasma nicotine, COT, 3HC); all were significant at P < 0.001 with the exception of the value marked as #, which was significant at P < 0.01.

*3HC data were available for a subset of the participants only.

### Table 4. Multiple linear regression models of the predictors of CPD, expired CO, and plasma COT

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>95% CI</th>
<th>Standardized β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma COT</td>
<td>0.15</td>
<td>0.11-0.19</td>
<td>0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Expired CO</td>
<td>0.09</td>
<td>0.03-0.15</td>
<td>0.12</td>
<td>0.004</td>
</tr>
<tr>
<td>Mentholated cigarettes BMI</td>
<td>−0.03</td>
<td>−0.06-0.00</td>
<td>−0.07</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**NOTE:** Nonmentholated cigarette users and males were coded as 0. Variables that were not normally distributed (CPD, expired CO, plasma COT, BMI) were log-transformed in the analyses.

**Abbreviation:** 95% CI, 95% confidence interval.
that this treatment-seeking sample may have attempted to stop smoking before the start of the trial; however, we excluded from these analyses any participants that reported smoking zero cigarettes within the past 7 days before the collection of biochemical data. That said, we cannot exclude the possibility that there may have been a selection bias as participants were light smokers who were highly motivated to quit smoking and had difficulty quitting in the past. Thus, they may be more dependent or smoke cigarettes differently from other non-treatment-seeking light smokers. In addition, participants may have underreported their cigarette consumption to meet the inclusion criteria for the clinical trial. The average plasma COT levels derived from *ad libitum* smoking (244 ng/mL) is similar to those found previously in a heavier smoking population of African-Americans (292 ng/mL) recruited for a clinical trial of smoking cessation at the same community health center as the current study, with an inclusion criteria of smoking at least 10 CPD (mean = 17 CPD; ref. 30). It is also possible that self-reported CPD is a poor measure of average cigarette consumption among individuals at this low level of smoking, which may in part explain their weak correlations to the biomarkers. In a previous study of an African-Canadian light-smoking population (median of 8 CPD), we found some discrepancies of cigarette consumption when it was reported as CPD, versus cigarettes per week, versus cigarettes per month. For example, one individual reported consuming 6 CPD, but 18 cigarettes per week and 60 cigarettes per month. Thus, in light smokers where daily smoking is variable and smoking may occur at irregular intervals, CPD may be a poor indicator of consumption and alternative measures of self-report, such as timeline follow-back procedures (43), needs to be tested. It is also notable that a large portion of the participants (45%) reported puffing or inhaling as far as the throat only. Although self-reported measures of depth of inhalation may not be representative of actual smoking behaviors (44), this may be another source of variability in cigarette exposure among these light smokers.

In summary, the results from this study suggest that the commonly used biomarkers of cigarette smoke exposure, expired CO and plasma COT, are significantly but weakly correlated with self-reported CPD. Furthermore, these relationships are not greatly altered by variables that were previously reported to have an influence on these parameters, such as CYP2A6 activity, smoking mentholated cigarettes, or age, although the relationships may differ slightly by gender and BMI. The proportion of variance in CPD explained by expired CO and plasma COT was generally lower than that observed in heavy Caucasians smokers even after accounting for these variables, suggesting these biomarkers are limited as indicators of cigarette smoke exposure among African-American light smokers.

Our study suggests that expired CO may be a poor indicator of smoking status as many smokers had expired CO levels below the traditionally defined cutoff level. Although plasma COT may be useful in ascertaining smoking status in this population, the level is highly influenced by the rate of CYP2A6 activity, and it is also a poor indicator of the levels of smoke exposure. This suggests the rate of CYP2A6 activity needs to be considered when COT is used as a biomarker of intake in African-American populations, where there are higher proportions of individuals with reduced rates of CYP2A6 activity compared with Caucasians. A number of other biomarkers such as thiocyanate or the tobacco alkaloids anabasine and anatabine have also been proposed (1); however, these also have their own set of limitations in terms of specificity, sensitivity, and cost for detection. Validated biomarkers are important for ascertaining smoking status before recruitment into research studies or clinical trials for smoking cessation, or for verifying abstinence among light smokers. In addition, validated biomarkers of cigarette smoke exposure are also necessary for the proper assessment of the dose-related risk of smoking and health outcomes in epidemiologic studies of African-Americans, a population that have been reported to have a disproportionately elevated risk of developing tobacco-related illnesses despite lower levels of self-reported cigarette consumption (45, 46).

**Disclosure of Potential Conflicts of Interest**

R. Tyndale: ownership interest, Nicogen; honoraria from speakers bureau, Novartis. J. Ahluwalia: honoraria from speakers bureau, Pfizer. N. Benowitz has been a paid expert witness in litigations against tobacco companies.

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**References**


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