Measures of Maternal Tobacco Exposure and Infant Birth Weight at Term

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This study was undertaken to determine the relation between self-reported number of cigarettes smoked per day and urine cotinine concentration during pregnancy and to examine the relations between these two measures of tobacco exposure and birth weight. Data were obtained from the Smoking Cessation in Pregnancy project, conducted between 1987 and 1991. Cigarette smoking information and urine cotinine concentration were collected for 3,395 self-reported smokers who were receiving prenatal care at public clinics in three US states (Colorado, Maryland, and Missouri) and who delivered term infants. General linear models were used to quantify urine cotinine variability explained by the number of cigarettes smoked per day and to generate mean adjusted birth weights for women with different levels of tobacco exposure. Self-reported number of cigarettes smoked per day explained only 13.9% of the variability in urine cotinine concentration. Birth weight declined as tobacco exposure increased; however, the relation was not linear. The sharpest declines in birth weight occurred at low levels of exposure. Furthermore, urine cotinine concentration did not explain more variability in birth weight than did number of cigarettes smoked. These findings should be considered by researchers studying the effects of smoking reduction on birth outcomes. Am J Epidemiol 2001;153:954–60.

Smoking during pregnancy is a preventable cause of reduced birth weight and is associated with preterm delivery and increased perinatal mortality (1–4). Unfortunately, tobacco exposure during pregnancy remains a major public health problem. Assuming there are 4 million births per year and that 20 percent of women smoke during pregnancy, as many as 800,000 infants are exposed in utero each year. Although a great deal of research has addressed the effects of tobacco exposure on fetal development, much about this relation remains unknown. For example, while there is agreement that smoking cessation before or early in pregnancy benefits the fetus (5, 6), it is less clear whether merely reducing the amount of cigarette smoking is also beneficial.

It is also unclear whether the effects of smoking on fetal development differ by race (7). Recently, scientists attempting to estimate the extent of fetal harm caused by tobacco exposure have used biomarkers such as cotinine to quantify tobacco exposure. Questions have been raised regarding the validity of such biomarkers as quantitative measures of exposure (8).

Previous studies have not addressed these issues adequately, because fundamental relations among different measures of tobacco exposure, as well as their relations to birth outcomes, have not been described fully. In this study, we used data from a smoking cessation intervention trial to examine the relation between two measures of tobacco exposure, self-reported number of cigarettes smoked per day and urine cotinine concentration, and to determine the functional relation between each measure of tobacco exposure and birth weight. We narrowed our study to women who delivered term infants to evaluate the effects of tobacco exposure on fetal growth independent of potential effects on preterm delivery.

MATERIALS AND METHODS

We used data from the Smoking Cessation in Pregnancy project, a prospective, randomized trial conducted from 1987 through 1991 that included 5,572 women enrolling in prenatal care in public prenatal clinics and in Women, Infants, and Children (WIC) programs in three US states (Colorado, Maryland, and Missouri). This federally funded project was conducted to evaluate the effect of low-intensity smoking cessation counseling on quitting behavior (9). Individual clinics were randomly assigned to provide their...
patients with newly developed written materials and brief smoking cessation messages or to provide their usual prenatal care.

All women attending study clinics for their first prenatal visit were screened for eligibility. Women who reported smoking even a puff of a cigarette within 7 days before thinking that they were pregnant or within 7 days before screening were considered smokers and were enrolled. Study subjects completed written questionnaires during the first or second prenatal visit (regardless of the gestational age), near the end of pregnancy (during the third trimester), and at the postpartum visit (6–12 weeks postpartum). During each of the three visits, a nurse abstracted additional information from subjects’ medical records. Variables collected at study enrollment included maternal age, race, education, parity, and prepregnancy body mass index; whether the woman had a husband or partner; and the state in which the clinic was located. Variables collected both at study enrollment and near the end of pregnancy included current number of cigarettes smoked per day, body mass index at the time of the visit, WIC enrollment, caffeine and alcohol consumption, and number of hours per day of passive smoke exposure.

Spot urine specimens were obtained for cotinine measurement within 2 days of the time the questionnaires were administered; urine was collected the same day that the questionnaire was completed for 97 percent of the patients and within 2 days for the remaining 3 percent. The number of hours between the last cigarette smoked and the collection of urine was not elicited. Additional details of the original study are presented elsewhere (9).

Subjects

For this analysis, we restricted the original study sample to women of White or Black race who delivered singleton term infants (37 or more completed weeks of gestation) of a plausible birth weight (between the 0.5 and 99.5 percentiles of birth weight (900–5,300 g)). In addition, these women had at least one self-reported estimate of the number of cigarettes smoked per day and a corresponding measurement of urine cotinine.

Birth weights were obtained from maternal interviews at the postpartum visit, when available. In Colorado and Missouri, data were merged with birth certificate records to obtain birth weights for infants of women who did not return for a postpartum visit. In Maryland, data were merged with the state’s Maternity Summary Form. For 81 percent of subjects, birth weights were obtained from maternal interview. For 76 percent of subjects, birth weight information was obtained from both sources; for 92 percent of these subjects, reported birth weights were within 30 g of those listed in vital statistics. Other measures of fetal growth such as head circumference were not collected in the original study and so were not included as outcome measures.

Urine cotinine testing

Urine specimens were frozen and then were shipped to the Centers for Disease Control and Prevention (Atlanta, Georgia) to be analyzed for cotinine (a metabolite of nicotine) by enzyme-linked immunosorbent assay (ELISA). Results of this procedure were validated by using a gas chromatography mass spectrometry method (10). We used a kinetic Jaffe reaction to measure true creatinine (10). The concentration of urine cotinine was adjusted for urine creatinine by using a regression method (11). We chose an active smoking threshold of 85 ng/ml adjusted for creatinine by examining the bimodal frequency distribution of the values for smokers and nonsmokers (10).

Data analysis

Relation between cigarette smoking and urine cotinine concentration. Data from women who reported that they were active smokers at the time that one or both urine specimens were obtained were used to explore the relation between urine cotinine concentration and number of cigarettes smoked per day. An active smoker was defined as any woman who reported smoking even one puff of a cigarette in the last 7 days. When two sets of corresponding measurements of reported number of cigarettes smoked and urine cotinine met this criterion, the one obtained at the latest visit during pregnancy was used. We limited this analysis to self-reported active smokers only to minimize the contribution of active smokers who falsely reported that they had quit. Using crude data, we generated a box-and-whiskers plot of corresponding values of number of cigarettes smoked per day and urine cotinine concentration.

We then used general linear models to quantify the amount of cotinine variability explained by self-reported number of cigarettes smoked per day. The measure of variability was \( r^2 \). We adjusted for factors potentially related to nicotine metabolism or smoking practices (such as depth of inhalation and brand preference). Adjustment variables included maternal age, education, body mass index at the time of the visit, state of residence, passive smoke exposure, and number of weeks of gestation. Because the clinics were randomly assigned in the original study, we also included a variable to measure the effects of clinic nested within intervention/control status. In these models, because the relation between urine cotinine concentration and reported number of cigarettes smoked per day was not linear, the log transformation of urine cotinine was used.

Tobacco exposure and birth weight. Data from women with corresponding measurements of tobacco exposure obtained in the third trimester (regardless of whether they reported actively smoking) were used to evaluate the relation between each measure of tobacco exposure and birth weight. Women not actively smoking were included in this analysis to generate a comparison group of “unexposed” infants. We used only exposure data obtained during the third trimester because research suggests that exposure to tobacco late in pregnancy has a greater effect on fetal growth than exposure early in pregnancy (6). When two sets of measurements of tobacco exposure were available from the third trimester, only the latest was used. We used general linear models to adjust for the major predictors of birth weight available in this data set: maternal age, race,
education, parity, prepregnancy body mass index, WIC enrollment, caffeine consumption, and alcohol consumption; presence of a husband or partner; US state in which the clinic was located; and the infant’s sex and gestational age. We again adjusted for clinic nested within intervention/control status. The two measures of tobacco exposure were divided into categories based on the distribution of women at different levels of exposure (0, <1, 1–2, 3–4, 5–9, 10, 11–19, 20, and 21–80 cigarettes smoked/day; 0–45, 46–85, 86–250, 251–500, 501–1,500, 1,501–2,500, 2,501–3,500, 3,501–4,500, 4,501–6,500, and 6,501–10,000 ng/ml of urine cotinine) and were plotted against mean adjusted birth weight. We used general linear models to quantify the amount of birth weight variability explained by categories of reported cigarette smoking and by urine cotinine concentration. In this case, because models in which the log transformation of cotinine was used did not explain as much of the variance in birth weight as categories of untransformed cotinine, untransformed cotinine values were used.

Race, cotinine, and birth weight. We used the t test to compare the mean cotinine concentration of Blacks and Whites who reported actively smoking at the time one or both urine specimens were obtained. General linear models were used to control for the number of cigarettes smoked per day and for factors potentially related to nicotine metabolism or smoking practices: maternal age, education, body mass index at the time of the visit, state of residence, and passive smoke exposure; number of weeks of gestation; and clinic nested within intervention/control status. We evaluated whether race affected the relation between urine cotinine concentration and birth weight by running additional models that included interaction terms for race by urine cotinine concentration.

The study proposal, consent form, and questionnaires were reviewed and were approved by the institutional review board of the Centers for Disease Control and Prevention.

RESULTS

Study population

Among the women screened, 5,572 reported smoking either just before thinking they were pregnant or at the time that screening was conducted. Of these women, 4,005 (71.9 percent) met the eligibility criteria (women of White or Black race who delivered singleton term infants of a plausible birth weight), and 3,395 (60.9 percent) had at least one self-reported estimate of the number of cigarettes smoked per day with a corresponding measurement of urine cotinine. Most women in this group were White, had at least a high school education, had a husband or partner present at the time of enrollment, and were multiparous. A total of 2,890 women in the study group (85.1 percent) reported actively smoking at the time one or both urine specimens were obtained, and 2,481 women (73.1 percent) had at least one set of corresponding measures of tobacco exposure obtained in the third trimester (table 1). The infant birth weight distribution of study subjects by level of tobacco exposure is shown in table 2.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2,943</td>
<td>86.7</td>
</tr>
<tr>
<td>Black</td>
<td>452</td>
<td>13.3</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>1,440</td>
<td>42.5</td>
</tr>
<tr>
<td>High school or more</td>
<td>1,946</td>
<td>57.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;19.8</td>
<td>975</td>
<td>28.7</td>
</tr>
<tr>
<td>19.8–26.0</td>
<td>1,514</td>
<td>44.6</td>
</tr>
<tr>
<td>26.1–29.0</td>
<td>263</td>
<td>7.7</td>
</tr>
<tr>
<td>&gt;29.0</td>
<td>341</td>
<td>10.0</td>
</tr>
<tr>
<td>Missing</td>
<td>302</td>
<td>8.9</td>
</tr>
<tr>
<td>Husband/partner present</td>
<td>2,546</td>
<td>75.4</td>
</tr>
<tr>
<td>Enrolled in WIC† program</td>
<td>1,124</td>
<td>33.3</td>
</tr>
<tr>
<td>State*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado</td>
<td>1,016</td>
<td>29.9</td>
</tr>
<tr>
<td>Maryland</td>
<td>1,179</td>
<td>34.7</td>
</tr>
<tr>
<td>Missouri</td>
<td>1,200</td>
<td>35.3</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>1,513</td>
<td>44.7</td>
</tr>
<tr>
<td>Active smoker by self-report at enrollment and/or in 3rd trimester</td>
<td>2,890</td>
<td>85.1</td>
</tr>
<tr>
<td>Questionnaire completed and urine obtained in 3rd trimester</td>
<td>2,481</td>
<td>73.1</td>
</tr>
<tr>
<td>Age (years) (mean (SD†))</td>
<td></td>
<td>22.8 (4.7)</td>
</tr>
<tr>
<td>Gestation at enrollment (weeks) (mean (SD))</td>
<td>18.6 (7.7)</td>
<td></td>
</tr>
<tr>
<td>Infant birth weight (g) (mean (SD))</td>
<td>3,269 (491.3)</td>
<td></td>
</tr>
<tr>
<td>Cigarettes per day (no.), smokers only (n = 2,890) (mean (SD))</td>
<td>11.5 (9.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Percentages do not total 100.0 because of rounding.
† WIC, Women, Infants, and Children; SD, standard deviation.

Relation between cigarette smoking and urine cotinine concentration

We examined the relation between self-reported number of cigarettes smoked per day and urine cotinine concentration for those 2,890 women who reported actively smoking at the time that one or both urine specimens were obtained. A box-and-whiskers plot of this relation is shown in figure 1. Among self-reported light smokers (<10 cigarettes/day), the median urine cotinine value increased steadily as cigarette smoking increased. However, cotinine concentrations reached a plateau at 15 cigarettes per day. At all levels of cigarette smoking, urine cotinine concentration varied considerably. For example, the urine cotinine concentrations of women who reported smoking 20 cigarettes per day ranged from 0 ng/ml to 10,000 ng/ml. The amount of variation in the log-transformed urine cotinine concentration explained
by self-reported number of cigarettes smoked per day during the third trimester was $r^2 = 13.9$ percent.

**Tobacco exposure and birth weight**

Mean adjusted birth weight decreased as the number of cigarettes smoked per day increased; however, the relation did not appear to be linear (figure 2). The sharpest decline in birth weight occurred at low levels of cigarette smoking. A similar pattern was found when urine cotinine values were used (figure 3). Third-trimester urine cotinine values did not explain more variation in birth weight than did third-trimester reported number of cigarettes smoked per day ($r^2 = 3.7$ percent and $r^2 = 4.0$ percent, respectively). After simultaneously controlling for the other factors described previously, we found that the full model explained less than 21 percent of the variability in birth weight for both cigarette smoking and urine cotinine concentration (20.4 and 20.1 percent, respectively).

**Race, cotinine, and birth weight**

Women who reported actively smoking smoked an average of 11.5 cigarettes per day. Black women who reported actively smoking smoked fewer cigarettes per day on average than White women did (7.4 vs. 12.1) and had a lower mean urine cotinine concentration (1,090 vs. 1,125 ng/ml). After adjusting for number of cigarettes smoked per day, we found that mean urine cotinine concentration was higher among Black women than White women (1,277 vs. 925 ng/ml, $p = 0.0001$). To evaluate the contribution of maternal factors to this association, we ran additional models that included maternal age, education, body mass index, state of residence, intervention status, and passive tobacco exposure as well as gestational age. The difference in mean urine cotinine was no longer significant (999 vs. 901 ng/ml, $p = 0.19$). Results of tests for interaction between race and urine cotinine concentration were not significant ($p = 0.19$). Hence, the degree to which birth

### TABLE 2. Distribution of study subjects by infant birth weight and self-reported maternal cigarette smoking in the third trimester ($n = 2,481$), Smoking Cessation in Pregnancy project, Colorado, Maryland, and Missouri, 1987–1991

<table>
<thead>
<tr>
<th>Birth weight (g)</th>
<th>No. of cigarettes/day</th>
<th>0</th>
<th>&lt;5</th>
<th>5–10</th>
<th>11–20</th>
<th>&gt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>&gt;3,600</td>
<td>190</td>
<td>38</td>
<td></td>
<td>88</td>
<td>22</td>
<td>166</td>
</tr>
<tr>
<td>3,400–3,599</td>
<td>84</td>
<td>17</td>
<td></td>
<td>59</td>
<td>15</td>
<td>111</td>
</tr>
<tr>
<td>3,200–3,399</td>
<td>84</td>
<td>17</td>
<td></td>
<td>66</td>
<td>16</td>
<td>142</td>
</tr>
<tr>
<td>3,000–3,199</td>
<td>65</td>
<td>13</td>
<td></td>
<td>78</td>
<td>20</td>
<td>120</td>
</tr>
<tr>
<td>&lt;3,000</td>
<td>73</td>
<td>15</td>
<td></td>
<td>107</td>
<td>27</td>
<td>271</td>
</tr>
<tr>
<td>Total</td>
<td>496</td>
<td></td>
<td>398</td>
<td>810</td>
<td>615</td>
<td>162</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Box-and-whiskers plot of the relation between urine cotinine and self-reported number of cigarettes smoked per day, Smoking Cessation in Pregnancy project, Colorado, Maryland, and Missouri, 1987–1991 ($n = 2,890$). For each category of reported cigarette smoking, the horizontal line dividing the box represents the median cotinine measurement with upper and lower hinges at the 75th and 25th percentiles. The whiskers represent the 90th and 10th percentiles, and outliers are depicted as separate points (plotted with a + symbol).
weight decreased with increasing urine cotinine concentration did not vary significantly by race.

**DISCUSSION**

In our study, self-reported number of cigarettes smoked per day and urine cotinine concentration were not closely correlated. Although birth weight was related to both the number of cigarettes smoked per day and urine cotinine levels, neither measure of exposure explained a substantial percentage of the variability in birth weight among term infants. Furthermore, although birth weight decreased as tobacco exposure increased, the relation between birth weight and tobacco exposure was not linear. Rather, the sharpest declines in birth weight were found at low levels of exposure. This pattern was found for both reported cigarette smoking and urine cotinine. Finally, although Black women had higher levels of urine cotinine than did White women with similar levels of self-reported cigarette smoking, the difference was not significant after we controlled for age,
body mass index, and other possible confounders. The rate of decrease in birth weight associated with urine cotinine concentration did not vary by race.

**Other studies**

Birth weight can be obtained easily and is a commonly selected neonatal outcome in studies attempting to estimate the amount of harm caused by tobacco exposure. Fetal tobacco exposure and its effects on birth weight may have long-term consequences as well. While the effect of in utero tobacco exposure on subsequent development of adult diseases is unknown, impaired fetal growth appears to be a predictor of coronary heart disease, hypertension, and impaired glucose tolerance later in life (12, 13). The exact nature of the functional relation between tobacco exposure and birth weight may help to direct research related to patterns of smoking behavior.

Many researchers currently report the effects of tobacco exposure on birth weight in terms of number of grams less per cigarette smoked or per nanogram of cotinine per milliliter of urine, implying that a linear relation exists (14–17). However, a sharp decline in birth weight at low levels of tobacco exposure was described in 1988 by Hebel et al., who used self-reported cigarette smoking (18), and more recently by Seeker-Walker et al., who used urine cotinine concentration (19). Our study, which has the advantage of a larger study population, confirmed these findings by using both measures of exposure. Thus, even low levels of tobacco exposure could have substantial effects on fetal growth. Furthermore, our data support the finding that the effects of tobacco exposure on infant birth weight plateau at moderate-to-heavy levels of exposure. Hence, reducing the number of cigarettes smoked during pregnancy without quitting may be of limited benefit for moderate and heavy smokers unless very low levels of smoking are achieved.

Previous studies have demonstrated a low correlation between biomarkers of tobacco exposure and self-reported cigarette smoking (20, 21). Possible explanations include inaccurate maternal reporting because of the stigma associated with smoking during pregnancy; rounding to fractions of packs of cigarettes smoked, and individual differences in inhalation, absorption, and metabolism (17, 22, 23). Jacob et al. found that even in a controlled environment, the correlation between nicotine use and urine cotinine concentration was only 0.62 ($R^2 = 38$ percent) (24). In our study, urine cotinine concentration varied greatly among women who reported similar levels of cigarette smoking, making it difficult to predict how much a woman smoked based on cotinine concentration, and vice versa. In some self-reported smokers, urine cotinine concentrations were below 85 ng/ml, the threshold for active smoking. Individual differences in how nicotine is metabolized may explain this observation. For example, nicotine is metabolized to cotinine by cytochrome P-450 2A6. Deletion alleles of the $CYP2A6$ gene (which produces cytochrome P-450 2A6) have been reported and can result in undetectable serum cotinine levels in active smokers (25).

In previous studies of the correlation between tobacco exposure and birth weight, biomarkers have not been substantially superior to self-report (14, 19, 20, 26). In our study of self-reported smokers, the birth weight variability explained by tobacco exposure was similarly low for both urine cotinine and number of cigarettes smoked per day. Self-report, although potentially inaccurate, is inexpensive and obtained readily. Collecting urine and measuring cotinine adds additional expense and inconvenience that may not be warranted in some settings.

Some studies suggest that cotinine concentration may be higher among Blacks than Whites even after adjustment for number of cigarettes smoked (21, 27). In our study, mean urine cotinine concentration was significantly higher in Black women than in White women reporting similar levels of cigarette smoking. However, the magnitude of this difference decreased and was no longer significant after adjustment for a number of potential confounders. Hence, factors such as maternal age and body mass index likely explain at least some of the racial differences observed in cotinine concentration. In addition, our findings lend support to existing evidence that the association between cotinine and birth weight does not vary by race (21). Thus, we recommend that smoking cessation programs continue to direct their efforts toward all pregnant smokers, regardless of race.

**Methodological considerations**

This study has several limitations. We lacked detailed information about the amount of time that elapsed between the last cigarette smoked and urine cotinine collection, and this limitation may have contributed to the poor correlation between these two measures of exposure. However, this problem likely affected mainly women who smoked irregularly or infrequently. In addition, we did not collect data about specific smoking practices, such as the type of cigarettes smoked and the depth of inhalation. Such factors could explain some of the elevation in cotinine concentrations found in Black women. Finally, there could be large disparities between reported and true levels of cigarette smoking that may have been unique to our study population. If so, this factor would limit the generalizability of our findings. However, even among women reporting high levels of cigarette smoking, the range of urine cotinine values was wide and included low concentrations of cotinine in some women. It is unlikely that women would falsely report high levels of cigarette smoking. Because we examined only urine cotinine, we could not draw conclusions about the relation between birth weight and serum or salivary cotinine concentrations.

Substantial loss to follow-up occurred in our study population. It is possible that an apparent nonlinear relation between tobacco exposure and birth weight was due to the loss of selected groups of women. For example, the sharp decline in birth weight at low levels of tobacco exposure could be an artifact resulting from the loss of women with low levels of tobacco use but whose infants were of relatively high birth weight. However, there is no obvious reason why that particular group of women would be disproportionately excluded. In addition, we did not have information about spontaneous abortions and so could not assess the
potential contribution of pregnancy losses to the infant birth weight distribution of our study group.

It is unlikely that cotinine itself directly affects fetal growth; rather, cotinine concentrations serve as a surrogate for nicotine exposure. Because we measured cotinine concentration in urine, and only a small fraction of cotinine is excreted unchanged by the kidneys (28), differences in urine cotinine concentration between races may merely reflect differences in cotinine metabolism and may not be closely related to levels of fetal nicotine exposure.

Conclusions

Ours is among the largest studies examining associations between self-reported cigarette smoking, urine cotinine concentration, and birth weight. We demonstrated a nonlinear relation between two measures of tobacco exposure and birth weight, a concept that may have important implications for pregnant women who reduce their tobacco use without quitting. In addition, our findings highlight some of the limitations of using self-reported cigarette smoking and urine cotinine to quantify tobacco exposure. Before we can determine how best to measure tobacco exposure, we need to learn more about which components of tobacco affect fetal development and what mechanisms are involved. Such knowledge could contribute to further improvements in infant health.

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