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Maternal Smoking during Pregnancy, Urine Cotinine Concentrations, and Birth Outcomes. A Prospective Cohort Study

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Wang X (Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA), Tager I B, Van Vunakis H, Speizer F E and Hanrahan J P. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. *International Journal of Epidemiology* 1997; **26:** 978–988.

Background. Most studies of the reproductive consequences of cigarette smoking base exposure on self-reported smoking habits. This study examines the relationship of birth outcomes to the timing and intensity of maternal active and passive smoking estimated both from self-reports and from cotinine concentration in maternal urine during early, middle, and late gestation.

Method. This cohort study included 740 white and Hispanic women who obtained antenatal care at the East Boston Neighborhood Health Center between 1986 and 1992. At each antenatal visit, information on maternal active and passive smoking was obtained by a detailed questionnaire, and by measurement of urine cotinine concentrations. Infant birth outcomes were obtained from hospital records. Multiple linear regression was used to evaluate antenatal smoking variables on birth outcomes, with adjustment for maternal demographic characteristics, reproductive history, alcohol use, maternal weight and height, and infant gender.

Results. The percentage of mothers who ever smoked cigarettes during pregnancy was 55.5% for white and 10.2% for Hispanic women. A significant inverse exposure-response relationship between cotinine concentration in maternal urine and infant size at birth was demonstrated. However, the relationship was less clear between maternal self-reported smoking status and these outcomes. For the entire gestation, a 1000 ng increase in mean urine cotinine concentration was associated with a 59 ± 9 g reduction in birthweight, a 0.25 ± 0.05 cm reduction in length, and a 0.12 ± 0.03 cm reduction in head circumference, respectively. For maternal passive smoking, the much smaller magnitude of effect precludes firm conclusions.

Conclusions. These data suggest that preventing and reducing active maternal smoking during pregnancy may have a beneficial impact on infant size at birth.

Keywords: birthweight, length, head circumference, cotinine, maternal smoking

In the US, a substantial proportion of women begin their pregnancies as smokers and continue to smoke during pregnancy.[1,2] Maternal smoking during pregnancy is known to be associated with fetal growth deficits:[3–5] infants born to smoking mothers are on average 200 g lighter and 1.4 cm shorter at birth than infants of non-smokers, even after adjustment for gestational age. Maternal cigarette smoking during pregnancy is also associated with increased risk of low birthweight, prematurity, spontaneous abortion, perinatal mortality, and ectopic pregnancy.[6–9] Evidence accumulated during the last decade suggests that antenatal maternal smoking has adverse long-term effects on the neurocognitive development of children.[10–16] Recent reports also suggest that antenatal smoking is associated with reduced pulmonary function in infancy[17] and childhood[18] and with adverse effects on postnatal height growth.[19] An even greater proportion of pregnant women are exposed passively to environmental tobacco smoke (ETS) than are active smokers. The question of whether maternal exposure to ETS also may be hazardous to the fetus has not been resolved,[20] although some studies suggest that such exposure is associated with reduced birthweight.[21]

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Epidemiological research on the reproductive consequences of cigarette smoking has been limited by several methodological difficulties.[8] Accurate assessment of exposure has been problematic, as most studies to date are based on self-reported smoking habits. The validity of these data depends on the subject's ability to accurately recall and report smoking information. Even if reporting is accurate, the relationship between the number of cigarettes smoked and the biological dose of toxic agents delivered to target sites is unknown. A number of factors, including the brand of cigarette smoked, the depth of inhalation, and individual differences in the uptake and metabolism of cigarette smoke components may all impact the actual level of fetal exposure. Thus, the self-reported number of cigarettes smoked daily may be an imprecise index of exposure. Quantification of exposure to ETS is even more difficult, in part because such exposure takes place in multiple locations. The contribution of each environment to total personal exposure varies with the amount of time spent and the concentration of tobacco smoke present. Misclassification of exposure may be an important reason for inconclusive results in studies of the effects of ETS exposure on birth outcomes.

Cotinine, the major metabolite of nicotine, is excreted primarily in the urine and has a much longer elimination half-life (12–36 h) than the parent compound.[22,23] Cotinine can provide a specific and objective measure of an individual's actual exposure to tobacco smoke to allow precise assessment of exposure-response relationships. Several published biochemical analyses of nicotine, cotinine, and thiocyanate in saliva, serum, urine, and hair have validated the relationships of these biomarkers to self-reported smoking habits.[24,25] However, only a few studies have examined these biochemical markers of tobacco smoke exposure in relation to pregnancy outcomes.[26–28]

The pathophysiological effects of maternal smoking on fetal growth may differ according to the timing and intensity of exposure, since the various fetal organ systems have distinct temporal patterns of growth and development.[29,30] An organ or system is probably most vulnerable to environmental insults during its period of most rapid growth. For example, fetal crown-to-heel length and head circumference appear to increase linearly through the second trimester and most of the third. By contrast, the slope of the fetal weight curve rises sharply during the third trimester, particularly after 30 weeks. Few studies have examined the relationship of birth outcomes to the amount of maternal smoking by the specific period of pregnancy.[9]

Intrauterine growth retardation (IUGR), one of the major consequences of antenatal smoking,[31] is manifested by reduced values for weight, length, and/or head circumference at birth, depending on the timing and intensity of the exposure. Few studies have examined the three growth indices simultaneously.

Because of public awareness of the adverse health effects of cigarette smoking and the decreasing prevalence of smoking, smokers have become increasingly distinguishable from non-smokers in terms of other factors relevant to birth outcomes. Specifically, smokers today have less education, lower income, higher alcohol consumption, and a less healthy lifestyle overall than non-smokers.[1,2,8] This observation underscores the importance of adequate adjustment for potential confounding factors in determining the health effects of smoking.

This study examined the timing and intensity of antenatal exposure to cigarette smoke in relation to infant weight, length, head circumference, and gestational age at birth in a large prospective cohort, accounting for other risk factors. The study evaluated self-reported active smoking and passive ETS exposure as well as the cotinine concentration in maternal urine as predictors of these outcomes.

METHODS

Study Population

The design of the study and the selection of the study population have been described previously.[32] Briefly, this is part of an ongoing longitudinal investigation on the health impact of antenatal exposure to cigarette smoke in infants and children being conducted at the East Boston Neighborhood Health Center (EBNHC), an ambulatory clinic in East Boston, Massachusetts. Between 25 March 1986 and 30 September 1992, a total of 2944 women presented to the clinic for antenatal care. Of these, 1336 women (45%) met the following enrolment criteria: (i) spoke English or Spanish, (ii) were older than 18 years of age, (iii) were in the first 20 weeks of gestation, and (iv) intended to use the EBNHC for paediatric care of their child. Eligible women were interviewed at their first antenatal clinic visit and given a description of the study and requirements for participation. Women who enrolled gave written consent. Of 1336 eligible women, 1000 agreed to participate (75%). This study was approved by the Human Subjects Committee of Brigham and Women's Hospital and Beth Israel Hospital in Boston and by a community Board of Directors of the EBNHC.

Upon enrolment, a detailed questionnaire was administered to each subject to solicit information on age, race, education, home characteristics, date of the last menstrual period (LMP), medical history, reproductive

history, current smoking status and smoking history, alcohol consumption, and the smoking practices of others present in the home.[17] Spirometry was performed and standing height was measured at the first or second antenatal visit. Maternal weight was measured at each antenatal visit.

At each subsequent antenatal visit, women were interviewed by means of a standard questionnaire that collected information on the brand and quantity of cigarettes smoked, exposure to other smokers in the home, and alcohol consumption since the previous visit. The subjects' medical records were reviewed to determine whether any complications of pregnancy, including hypertension, gestational diabetes, urinary tract infection, or vaginal bleeding, had been noted at the visit by the examining obstetrician. After participating women had given birth, maternal and infant hospital records were reviewed to obtain data on complications of delivery, antenatal fetal distress, postdelivery hospital stay, and the necessity for supplemental oxygen for the baby. Weight, length, and head circumference at birth and Apgar scores at 1 min and 5 min were recorded for all infants, as was information on breast versus bottle feeding.

In this study, gestational age at birth was defined as the interval between the first day of the mother's LMP and the date of birth. Because of post-conception bleeding or menstrual irregularities, the presumed date of LMP may be in error. No data are available on whether there are systematic differences in the reporting of LMP between smoking and non-smoking women.

Biochemical Assay of Urine Cotinine Concentration

At each antenatal visit, a urine specimen was collected for measurement of the cotinine concentration. At birth, the mother's urine and blood as well as the infant's cord blood were obtained for the same purpose. Specimens were stored at 4°C. Cotinine was measured in urine and serum by radioimmunoassay.[33,34] The minimal detection limit was 3 ng/ml, with a coefficient of variation of 10%. Creatinine was measured in urine by an automated Jaffe reaction method.[35] Urinary cotinine measurements were corrected for the urinary creatinine concentration with the method proposed by Thompson *et al.*[36] to account for intersubject variations in urinary dilution.

Smoke Exposure Classification

In the initial descriptive analysis, mothers were grouped into one of the three categories on the basis of their self-reported smoking behaviour during pregnancy: non-smokers, continuous smokers (classified as smokers at all antenatal visits), or intermittent smokers (starter, quitter, or both). In the subsequent multivariate analysis, intrauterine exposures to cigarette smoke were characterized further for each of three intervals during pregnancy ($<$18 weeks, 18–31 weeks, and $>$31 weeks of gestation) and for the entire pregnancy. For each pregnancy interval, self-reported smoking amount and cotinine concentration in maternal urine were evaluated as separate indicators of exposure. Self-reported smoking amount was analysed as a continuous variable (number of cigarettes per day) and as a categorical variable (0, 1–4, 5–9, 10–14, 15–19, and $\geqslant$20 cigarettes per day). The cotinine concentration in maternal urine was also analysed as a continuous variable (expressed as per 1000 ng increase) and as a categorical variable (0–30, 31–100, 101–1000, 1001–2500, 2501–5000, $>$5000 ng). These categories were chosen on the basis of dose-response relationship and adequate sample size in each category. Women were grouped into three categories with regard to their passive exposure to ETS at home: unexposed, exposed to one or more smokers, and unknown exposure status. This classification was based on the mother's report of active smokers living in the home.

Definition of Other Risk Factors

Maternal alcohol consumption during pregnancy was defined for each of the three intervals delineated above ($<$18 weeks, 18–31 weeks, and $>$31 weeks of gestation) and for the entire pregnancy. For each interval, maternal self-reported alcohol use was analysed as a categorical variable (non-users and quartiles of alcohol use among users).

Other potential risk factors or confounders evaluated in the analysis include maternal race (white or Hispanic), maternal age ($<$20, 20–29, 30–34, or $\geqslant$35 years), maternal education ($<$12, 12, or $>$12 years), parity (1, $\geqslant$2), fetal gestational age at entry into the study ($<$13, 13–17, or $\geqslant$18 weeks), history of premature birth (yes or no), history of stillbirth (yes or no), any complication of the current pregnancy (yes or no), maternal weight (lbs) at enrolment, maternal height (inches), and infant's sex. This study used maternal weight at enrolment (after adjusting for gestational age at entry) as a proxy for pre-pregnancy weight as the latter is not available.

Statistical Methods

The major outcomes examined were weight, length, and head circumference at birth. These outcomes were analysed as both original scales (e.g. birthweight in grams) and Z-scores. For each infant, the Z-score for each outcome is calculated as:

$$\text{Z-score} = (O - P)/SD$$

where O is the infant's observed birthweight, length, or head circumference, P is the mean value of all infants born at the given week of gestation, SD is the standard deviation of the mean. This Z-score transformation eliminates the influence of gestational age on these outcomes and stabilizes the variances over the range of gestation. Furthermore, the effects of smoking can be compared among the birth outcomes. The Z-score for gestational age was defined as the difference between the observed value and the mean value divided by its standard deviation.

Multiple linear regression was used to evaluate the antenatal smoking variables individually and jointly as predictors of weight, length, head circumference, and gestational age at birth, with adjustment for all other covariates listed above. Regression parameters were estimated by the ordinary least squares method. Graphical and residual analyses were performed to assess modelling assumptions. Potential interactions of smoking with alcohol use and race were evaluated. The results were insignificant. Analyses also were stratified by race. Differences in the estimates between racial groups were compared by two-tailed t-test. Again, the results were insignificant. Since maternal weight itself may be affected by cigarette smoking, multiple regression analyses were performed with and without maternal weight in the model. The results were similar.

RESULTS

This study was limited to the 407 white and 333 Hispanic mothers with singleton live births. Twenty-two per cent of the subjects entered the study before 13 weeks of gestation, 46% between 13 and 17 weeks of gestation, and 32% between 17 and 20 weeks of gestation. Infants' gestational ages at birth ranged from 30 to 45 weeks. The mean numbers of antenatal clinic visits and of maternal urine specimens obtained were 6.2 (SD = 1.6) and 5.7 (SD = 1.9), respectively. These characteristics did not differ significantly by maternal smoking status. However, the percentage of mothers who ever smoked cigarettes during pregnancy was much higher for white women than for Hispanic women (55.8% versus 10.2%). As shown in Table 1, mothers who reported continuous smoking during pregnancy tended to have a lower level of attained education than non-smoking mothers. Furthermore, smoking mothers were more likely to drink alcohol and to be passively exposed to ETS at home than their non-smoking counterparts. The notion that smokers tend to be taller and heavier is at least partially due to the fact that smokers are more likely to be white than are non-smokers.

TABLE 1 *Maternal characteristics by maternal active smoking status during pregnancy*

Maternal characteristic	None N = 483	Continuous N = 164	Intermittent N = 93
Race (%)			
White	38.1	92.7	76.3
Hispanic	61.9	7.3	23.7
Age years (%)			
<20	4.4	4.3	5.4
20–29	71.2	68.3	74.2
30–34	17.6	16.5	16.1
⩾35	6.8	11.0	4.3
Education years (%)			
<12	40.2	40.2	32.6
12	31.9	43.3	47.8
>12	27.9	16.5	19.6
Parity ⩾2 (%)	58.1	62.0	51.1
History of stillbirth (%)			
Primiparous	0.0	0.0	0.0
Multiparous	2.1	2.0	2.1
All	1.2	1.2	1.1
History of prematurity (%)			
Primiparous	–	–	–
Multiparous	3.2	3.0	0.0
All	1.9	1.8	0.0
Pregnancy complication (%)			
Primiparous	18.3	19.4	20.0
Multiparous	13.4	14.9	20.8
All	15.6	16.6	20.4
Height (inches)			
Mean ± SD	62.3 ± 2.7	63.7 ± 2.3	63.1 ± 3.3
Weight at entry (lbs)			
Mean ± SD	145.4 ± 32.5	151.8 ± 35.9	151.8 ± 36.8
Alcohol use during pregnancy (%)	32.7	46.3	48.4
Passive smoking (%)	15.1	49.0	34.0

The relationships among maternal self-reported active smoking, self-reported passive exposure to ETS, and urinary cotinine concentration over the entire period of pregnancy are shown in Figure 1. The mean creatinine-corrected urine cotinine concentrations were 20 ng (95% confidence interval [CI] : 18.4–21.6) in mothers who were neither active nor passive smokers and 41 ng (95% CI : 35–47) for non-smoking mothers who were passively exposed to ETS at home. The difference was statistically significant ($P < 0.001$). Among non-smoking mothers of unknown ETS exposure status, the mean concentration was 28 ng (95% CI : 25–31). The mean cotinine value was 962 ng (95% CI : 700–1224) in intermittent smokers. Much higher levels were observed for continuous smokers. Among women classified as smokers at all antenatal clinic visits, there was an approximately linear relationship between the mean urinary cotinine concentration and the mean reported

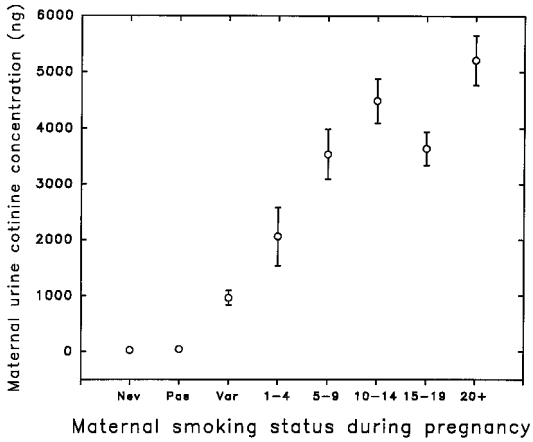

FIGURE 1 *Maternal self-reported active and passive smoking status and urinary cotinine concentration over the entire period of pregnancy, East Boston Cohort. Nev = non-smokers; Pas = passive maternal exposure to environmental tobacco smoke; Var = intermittent smokers; continuous smokers are grouped according to the number of cigarettes smoked per days (the groups are mutually exclusive)*

TABLE 2 *The mean and standard deviation of birth outcomes by maternal active smoking status during pregnancy*

Birth outcomes	Mean ± SD values for indicated maternal smoking status during pregnancy		
	None	Continuous	Intermittent
Gestation, weeks			
All	39.9 ± 2.4	40.1 ± 2.6	40.4 ± 2.3
White	40.3 ± 2.1	40.0 ± 2.5	40.5 ± 2.5
Hispanic	39.7 ± 2.6	40.3 ± 3.5	40.1 ± 1.9
Birthweight, g			
All	3447 ± 516	3190 ± 548	3391 ± 562
White	3529 ± 483	3192 ± 552	3406 ± 587
Hispanic	3395 ± 530	3167 ± 516	3344 ± 479
Length, cm			
All	50.5 ± 2.8	49.3 ± 3.0	50.6 ± 3.1
White	50.7 ± 2.9	49.3 ± 3.0	50.7 ± 3.4
Hispanic	50.4 ± 2.7	49.7 ± 2.3	50.3 ± 1.9
Head circumference, cm			
All	34.5 ± 1.6	34.0 ± 1.8	34.4 ± 1.5
White	34.6 ± 1.6	33.9 ± 1.8	34.4 ± 1.5
Hispanic	34.5 ± 1.6	34.2 ± 1.6	34.2 ± 1.4

number of cigarettes smoked per day, up to 14 cigarettes/day. Among women who reported smoking more than 14 cigarettes/day, this relationship was less consistent. At birth, cotinine levels in infant cord-blood serum were highly correlated with maternal cotinine concentrations in both serum ($r = 0.91$, $P < 0.001$) and urine ($r = 0.72$, $P < 0.001$).

For each of the three gestational intervals, cotinine concentrations in maternal urine were also significantly correlated with the reported number of cigarettes smoked. The correlation coefficients range from 0.68 to 0.73 ($P < 0.001$). In addition, maternal smoking between intervals of gestation were highly correlated with each other ($r = 0.63$–0.78, $P < 0.001$) both for self-reported cigarettes smoked per day and for urine cotinine concentration. Among smokers, non-Hispanic white mothers smoked more heavily than Hispanic mothers (mean: 14 versus 7 cigarettes per day for continuous smokers). However, the relationship between maternal self-reported smoking amount and urinary cotinine concentration did not differ for these groups.

Table 2 presents the crude relationship between maternal smoking status during pregnancy and birth outcomes. Compared with infants born to non-smoking mothers, those born to continuously smoking mothers were, on average, 257 g lighter, 1.2 cm shorter, and 0.5 cm smaller in head circumference. Compared with infants of non-smokers, infants born to intermittent smokers were 56 g lighter in birthweight, but had similar birth length and head circumference. These patterns were similar for whites and Hispanics, but the magnitude of the differences was slightly greater for whites.

Mean residuals of Z-scores from regression analyses of infant weight, length, and head circumference at birth on maternal urinary cotinine concentration and maternal self-reported cigarette smoking habits over the entire pregnancy are plotted in Figures 2 and 3, respectively. The prediction models used to obtain the residuals included maternal race, age, education, and parity; fetal gestational age at entry into the study; history of premature birth; history of stillbirth; complication of the current pregnancy; maternal weight at first visit; maternal height; maternal alcohol consumption during pregnancy; and infant's sex. To make the data clinically meaningful, the opposite axis of the Z-scores give the corresponding values on the original scales (e.g., –0.2 in Z-score of birthweight is equivalent to 3249 g). The data show a clear inverse exposure-response relationship between maternal urinary cotinine concentration and each of the three birth outcomes (Figure 2), but this relationship was not as clear for maternal self-reported cigarette smoking (Figure 3). No racial differences in the effects of maternal cigarette smoking were evident.

The above associations were further evaluated to ascertain the effects of the timing of exposures during pregnancy. As stated above, mean urinary cotinine

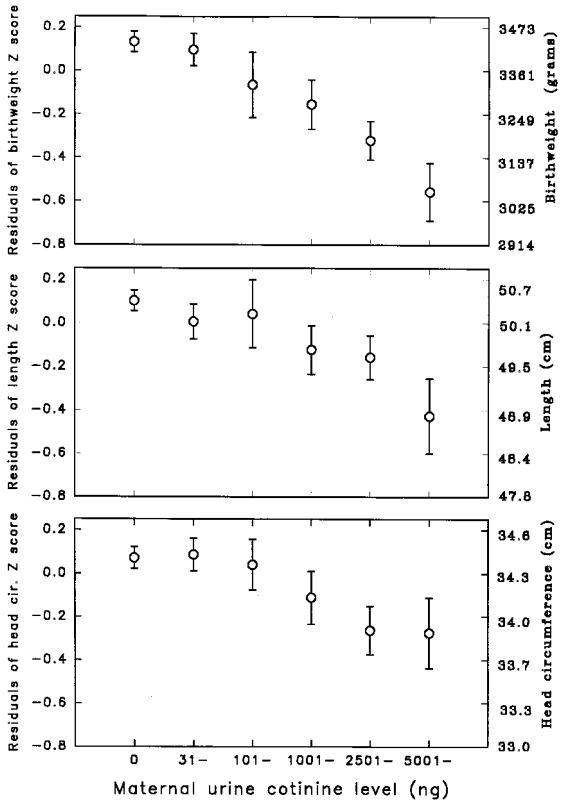

FIGURE 2 *Mean residuals of weight, length, and head circumference at birth by maternal urinary cotinine concentration over the entire period of pregnancy, East Boston Cohort*

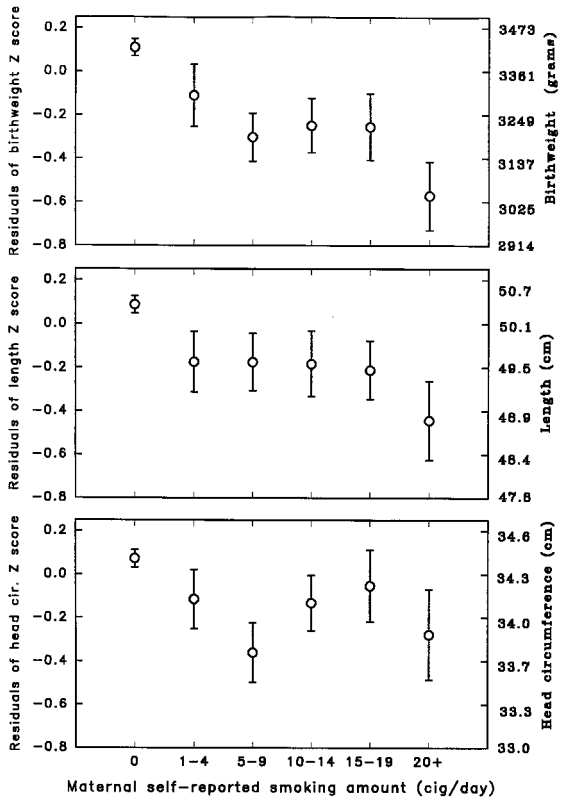

FIGURE 3 *Mean residuals of weight, length, and head circumference at birth by maternal self-reported active smoking status over the entire period of pregnancy, East Boston Cohort*

levels were evaluated for each of the three intervals of gestation (<18, 18–31, ⩾31 weeks) and for the entire pregnancy; for each pregnancy interval, urinary cotinine level was analysed as both a categorical and a continuous variable (expressed as per 1000 ng). Since no significant racial differences in the effect estimates were found, only common estimates for both whites and Hispanics are presented. Table 3 presents regression results on birth outcomes expressed as Z-scores. When smoking in each of the three intervals was modelled individually, maternal smoking in each interval was significantly associated with reductions in weight, length, and head circumference at birth. However, the estimates of the effects were somewhat smaller for early exposure (<18 weeks) and greater for midterm exposure (18–31 weeks). Only early and midterm exposures were significantly associated with gestational age at birth.

Multiple linear regression analysis also was performed on the birth outcomes in the original scales (Table 4). The models were the same except for addition of gestational age and its quadratic term. In contrast to Table 3, the regression coefficients have straightforward meaning, e.g. for the entire gestation, each 1000 ng increase in mean urine cotinine concentration is associated with a 59 ± 9 g reduction in birthweight, a 0.25 ± 0.05 cm reduction in length, and a 0.12 ± 0.03 cm reduction in head circumference. The pattern of associations for each pregnancy interval were similar to those shown in Table 3.

DISCUSSION

Most studies of reproductive consequences have based analysis of exposure on self-reported smoking habits, a subjective and potentially unreliable index of dose. In a study of pregnant women in Maine, Haddow *et al.*[26] found that serum cotinine level was a better predictor of birthweight than the self-reported number of cigarettes smoked per day. English *et al.*[27] studied a cohort of

TABLE 3 *Effects[a] of cotinine concentrations in maternal urine on birth outcomes expressed in terms of Z-scores, by the timing of exposure during pregnancy*

Timing of exposure Cotinine (ng)	Z-scores (Beta ± SE) for indicated birth outcomes			
	Weight (g)	Length (cm)	Head circumference (cm)	Gestational age (weeks)
<18 wks				
31–100	–0.17 ± 0.11	–0.03 ± 0.12	0.07 ± 0.12	–0.01 ± 0.12
101–1000	–0.56 ± 0.17**	–0.22 ± 0.17	–0.24 ± 0.18	0.03 ± 0.18
1001–2500	–0.32 ± 0.15*	–0.48 ± 0.15**	–0.17 ± 0.16	–0.08 ± 0.16
2501–5000	–0.65 ± 0.14**	–0.27 ± 0.15	–0.37 ± 0.15*	–0.25 ± 0.15
5000+	–0.75 ± 0.17**	–0.44 ± 0.18*	–0.50 ± 0.18**	–0.35 ± 0.19
Per 1000	–0.09 ± 0.02**	–0.07 ± 0.02**	–0.06 ± 0.02**	–0.04 ± 0.02*
18–31 wks				
31–100	–0.06 ± 0.10	–0.10 ± 0.10	–0.07 ± 0.10	–0.29 ± 0.10**
101–1000	–0.13 ± 0.17	–0.01 ± 0.18	–0.09 ± 0.18	0.00 ± 0.18
1001–2500	–0.39 ± 0.13**	–0.35 ± 0.14*	–0.26 ± 0.14	0.02 ± 0.14
2501–5000	–0.68 ± 0.13**	–0.35 ± 0.13**	–0.38 ± 0.14**	–0.38 ± 0.14**
5000+	–0.83 ± 0.15**	–0.59 ± 0.16**	–0.59 ± 0.16**	–0.37 ± 0.16*
Per 1000	–0.12 ± 0.02**	–0.08 ± 0.02**	–0.08 ± 0.02**	–0.04 ± 0.02*
32+ wks				
31–100	–0.08 ± 0.10	–0.14 ± 0.10	–0.05 ± 0.11	–0.04 ± 0.10
101–1000	–0.26 ± 0.16	–0.35 ± 0.17*	–0.16 ± 0.17	–0.01 ± 0.16
1001–2500	–0.41 ± 0.14**	–0.45 ± 0.15**	–0.15 ± 0.15	–0.01 ± 0.15
2501–5000	–0.66 ± 0.14**	–0.46 ± 0.14**	–0.57 ± 0.15**	–0.21 ± 0.14
5000+	–0.74 ± 0.16**	–0.56 ± 0.17**	–0.40 ± 0.17*	–0.14 ± 0.17
Per 1000	–0.11 ± 0.02**	–0.08 ± 0.02**	–0.06 ± 0.02**	–0.01 ± 0.02
Entire gestation				
31–100	–0.13 ± 0.09	–0.17 ± 0.10	–0.04 ± 0.10	–0.19 ± 0.10
101–1000	–0.34 ± 0.16*	–0.15 ± 0.16	–0.11 ± 0.17	0.09 ± 0.17
1001–2500	–0.47 ± 0.13**	–0.35 ± 0.14*	–0.29 ± 0.14*	0.12 ± 0.14
2501–5000	–0.66 ± 0.12**	–0.40 ± 0.13**	–0.45 ± 0.13**	–0.40 ± 0.13**
5000+	–0.96 ± 0.16**	–0.72 ± 0.17**	–0.51 ± 0.17**	–0.25 ± 0.17
Per 1000	–0.12 ± 0.02**	–0.09 ± 0.02**	–0.07 ± 0.02**	–0.03 ± 0.02

[a] The effects were estimated by multiple linear regressions with adjustments for confounders. Maternal urinary cotinine concentration was analysed as a categorical and a continuous variable (per 1000 ng).
* $P < 0.05$, ** $P < 0.01$.

women and their offspring enrolled at Kaiser Permanente Hospital facilities in Oakland, California. They reported that black women had higher cotinine levels at each self-reported smoking dose than white women. However, no significant racial differences in the degree of decrease in birthweight per unit of cotinine were found.

In the current study, the effects of antenatal smoking on birth outcomes were assessed on the basis of both maternal self-reports of active and passive smoking and prospective serial measurements of maternal urinary cotinine levels during pregnancy. A significant exposure-response relationship was evident between a high cotinine concentration in maternal urine and reduced infant weight, length, and head circumference at birth (Figure 2) but this was less clear when maternal self-reports of smoking were used (Figure 3). The stronger exposure-response relationship for cotinine concentrations suggests that this objective measure more accurately represents the individual differences in smoking behaviour (e.g. depth of inhalation, brand of cigarette, and uptake and metabolism of smoke constituents). These data indicate that misclassification of fetal exposure was more likely in the absence of urinary cotinine assays, despite a prospective design and intensive quantitative exposure ascertainment by repeated self-reporting of smoking behaviour. Exposure misclassification may obscure significant associations between antenatal smoking and birth outcomes.

The gestational age at which a fetus is exposed to the constituents of cigarette smoke may influence the degree to which and the manner in which birth outcomes are affected. Limited data are available on the timing of

TABLE 4 *Effects*[a] *of cotinine concentrations in maternal urine on infant birth outcomes on untransformed scales, by the timing of exposure during pregnancy*

Timing of exposure Cotinine (ng)	Beta ± SE for indicated birth outcomes			
	Weight (g)	Length (cm)	Head circumference (cm)	Gestational age (weeks)
<18 weeks				
31–100	–84 ± 52	–0.26 ± 0.32	0.11 ± 0.18	–0.02 ± 0.29
101–1000	–267 ± 79**	–0.76 ± 0.48	–0.30 ± 0.27	0.08 ± 0.44
1001–2500	–150 ± 69*	–1.53 ± 0.42**	–0.23 ± 0.24	–0.19 ± 0.38
2501–5000	–322 ± 65**	–0.85 ± 0.40*	–0.59 ± 0.23*	–0.60 ± 0.37
5000+	–343 ± 81**	–1.18 ± 0.50**	–0.71 ± 0.28**	–0.85 ± 0.46
Per 1000	–42 ± 9**	–0.18 ± 0.06**	–0.09 ± 0.03**	–0.11 ± 0.05*
18–31 weeks				
31–100	–23 ± 46	–0.35 ± 0.28	–0.06 ± 0.16	–0.71 ± 0.25**
101–1000	–79 ± 79	–0.13 ± 0.49	–0.07 ± 0.27	0.00 ± 0.44
1001–2500	–189 ± 63**	–1.11 ± 0.39*	–0.39 ± 0.22	0.06 ± 0.35
2501–5000	–321 ± 61**	–1.01 ± 0.37**	–0.62 ± 0.21**	–0.93 ± 0.34**
5000+	–386 ± 72**	–1.62 ± 0.44**	–0.88 ± 0.25**	–0.90 ± 0.40*
Per 1000	–55 ± 9**	–0.21 ± 0.05**	–0.13 ± 0.03**	–0.11 ± 0.05*
32+ weeks				
31–100	–44 ± 47	–0.44 ± 0.29	–0.08 ± 0.16	–0.09 ± 0.25
101–1000	–126 ± 76	–1.09 ± 0.46*	–0.19 ± 0.26	–0.03 ± 0.40
1001–2500	–205 ± 68**	–1.45 ± 0.41**	–0.24 ± 0.23	–0.02 ± 0.35
2501–5000	–298 ± 64**	–1.23 ± 0.39**	–0.86 ± 0.22**	–0.52 ± 0.34
5000+	–362 ± 77**	–1.46 ± 0.47**	–0.62 ± 0.26*	–0.33 ± 0.40
Per 1000	–51 ± 9**	–0.21 ± 0.05**	–0.10 ± 0.03**	–0.03 ± 0.05
Entire gestation				
31–100	–57 ± 44	–0.52 ± 0.27	–0.03 ± 0.15	–0.46 ± 0.25
101–1000	–169 ± 74*	–0.57 ± 0.46	–0.10 ± 0.25	0.22 ± 0.40
1001–2500	–235 ± 62**	–1.17 ± 0.38*	–0.48 ± 0.21*	0.30 ± 0.34
2501–5000	–308 ± 58**	–1.11 ± 0.36**	–0.71 ± 0.20**	–0.97 ± 0.32**
5000+	–454 ± 77**	–1.99 ± 0.47**	–0.76 ± 0.26**	–0.60 ± 0.42
Per 1000	–59 ± 9**	–0.25 ± 0.05**	–0.12 ± 0.03**	–0.06 ± 0.05

[a] The effects were estimated by multiple linear regressions with adjustments for confounders. Maternal urinary cotinine concentration was analysed as a categorical and a continuous variable (per 1000 ng). Weight in grams, length and head circumference in centimeters, and gestational age in weeks. * $P < 0.05$, ** $P < 0.01$.

exposure and birth outcomes. Lieberman *et al.*[9] recently studied the timing of fetal exposure to maternal smoking (based on self-reports) and low birthweight at term. The authors suggested that smoking during the third trimester retarded fetal growth more significantly than smoking earlier in pregnancy. To our knowledge, no study has examined the effects of maternal smoking stratified by the specific intervals of exposure during pregnancy using an objective exposure measure such as cotinine.

In this study, the timing of fetal exposure to cigarette smoke was characterized by separate analysis of four periods: <18 weeks, 18–31 weeks, >31 weeks of gestation; and the entire pregnancy. As expected, maternal smoking variables were highly correlated from one gestational age period to another. When modelled individually, each antenatal smoking variable was significantly associated with reduced weight, length, and head circumference at birth. However, the estimates of the effects were somewhat smaller for early exposure (<18 weeks) and greater for midterm exposure (18–31 weeks). More detailed analysis was not possible due to the problem of colinearity and constraints imposed by the sample size.

Studies examining the reproductive consequences of passive exposure to ETS have so far yielded mixed results.[20,21] In this study, the mean cotinine concentration significantly differs between non-smokers and passive smokers, 20 ng (95% CI : 18.4–21.6) versus 41 ng (95% CI : 35–47). It provides evidence that

women passively exposed to ETS have measurable increases of a biomarker of cigarette smoke. Furthermore, the data show small but detectable negative effects on infant weight, length, and head circumference at birth when the maternal urinary cotinine level is as low as 31–100 ng/mg of creatinine (a range found in passively exposed women) compared to never smoking low cotinine group (<31 ng). However, the results are only suggestive but not conclusive. These findings are consistent with a report on hair concentrations of nicotine and cotinine in 94 mother-infant pairs[24] and a report on serum cotinine levels in 1231 non-smoking women in relation to birthweight.[28]

Few data are available to explain the observed difference between the effects of maternal and paternal smoking on offspring.[18] This investigation provides biochemical evidence that maternal smoking during pregnancy results in fetal exposures to the constituents of tobacco smoke that are at least 30-fold and probably 100-fold higher than exposure levels resulting from ETS (passive smoking) alone. A reasonable inference from this evidence is that maternal smoking during pregnancy results in much higher levels of fetal exposure to the toxic constituents of tobacco smoke than does paternal smoking or any other source of passive ETS exposure. This evidence strongly supports previous speculation that active maternal smoking during pregnancy is likely to have more pronounced and longstanding effects on fetal growth than passive ETS exposure.[17,18]

Head circumference has often been regarded (along with weight and length) as a somatic size outcome at birth. Recent recommendations have included microcephaly as a criterion of central nervous system dysfunction in the description of fetal alcohol syndrome.[37] Hack *et al*. found that among infants with very low birthweight subnormal head circumference at 8 months of age is associated with poor cognitive function, academic achievement, and behaviour at 8 years of age.[38] In this study, infants of smoking mothers had a significantly smaller head circumference at birth than those of non-smoking mothers, even after adjustment for other risk factors. In addition, there was a significant exposure-response relation between maternal antenatal urinary cotinine concentration and infant head circumference at birth. These data support the hypothesis that *in utero* exposure to cigarette smoke may adversely affect brain growth, as evidenced by reduced head circumference at birth.

Data on long-term growth and developmental sequelae of antenatal maternal smoking is accumulating.[10–19] The extent to which the effects of *in utero* exposure to cigarette smoking persist beyond infancy remain in question. A recent study[19] suggests that children whose mothers were heavy smokers during pregnancy are of shorter stature at age 5 years than children of non-smokers. This effect appears to be due to *in utero* exposure rather than postnatal ETS exposure.

Several limitations of this study could have influenced the results. The sample included only women who made at least one antenatal clinic visit and enrolled in the study before 20 weeks of gestation. Thus, women who received late or no antenatal care were excluded; in this and other populations such women are more likely to smoke during pregnancy and are at higher risk of adverse pregnancy outcomes. No attempt was made to assess or measure exposure to other substances, such as marijuana or cocaine, known from previous investigations to adversely affect fetal growth.[39] However, the magnitude of the effect reported for these substances was much smaller than that observed for cigarette smoke in the current investigation.

In summary, we assessed the effects of antenatal smoking on birth outcomes on the basis of both maternal self-reports of active and passive smoking and prospective serial measurements of maternal urinary cotinine levels during specific periods of pregnancy. The data demonstrate a significant inverse exposure-response relationship between the antenatal cotinine concentration in maternal urine and infant weight, length, and head circumference at birth, even after adjustment for other variables known to influence these birth outcomes. However, this relationship was not as clear for maternal self-reported cigarette smoking. This study can not adequately assess the timing of fetal exposure to cigarette smoke in relation to birth outcomes due to the problem of colinearity and constraints imposed by the sample size. Infants born to non-smoking mothers who were passively exposed to ETS during pregnancy were measurably exposed to cigarette smoke and manifested small but consistent negative effects on birth outcomes. Nevertheless, these results are suggestive, but not conclusive. We speculate that preventing and reducing active smoking among women of reproductive age is likely to have a significant beneficial impact on children's growth and development.

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