Parental tobacco smoking is associated with lower airway function and an increased incidence of wheezy respiratory illnesses in infants. We evaluated in 76 healthy infants whether exposure to parental tobacco smoking was associated with airway hyperreactivity, which could contribute to lower airway function and the increased wheezy illnesses. Airway function was measured using the raised-volume rapid thoracic compression technique, and airway reactivity was assessed by methacholine challenge (0.015–10 mg/ml), which was stopped for a more than 30% decrease in forced expiratory flow (FEF)75, or the final dose with a less than 30% decrease. Parental tobacco smoking was associated with lower baseline airway function (FEF75, 600 vs. 676 ml/second, p < 0.04; FEF25–75, 531 vs. 597 ml/second, p < 0.05). Infants exposed to tobacco smoking were approximately half as likely to develop a more than 30% decline in FEF75 at any given methacholine dose (hazard ratio = 0.4, p = 0.001). In addition, a history of asthma in an extended family member increased the likelihood that an infant would develop a more than 30% decline in FEF75 (hazard ratio = 1.7, p = 0.04). We conclude that exposure to parental smoking is associated with lower airway function but not increased airway reactivity; however, family history of asthma is associated with heightened airway reactivity.

**Keywords:** airway function; bronchial reactivity; cotinine; tobacco smoke exposure

Parental tobacco smoking is associated with increased respiratory symptoms in school-age children, as well as disease severity in children with asthma (1–2). Several studies have reported that in older children with asthma, parental tobacco smoking is also associated with heightened airway reactivity, which is a phenotypic characteristic of asthma (1–3). However, it remains unclear whether exposure to tobacco smoking, particularly early in life, is causally related to the development of airway hyperreactivity or a trigger for asthma symptoms in subjects with airway hyperreactivity (3).

Parental tobacco smoking is associated with an increased incidence of wheezing and lower respiratory illnesses in infants and toddlers (4–6), as well as lower airway function than infants not exposed to tobacco smoking, even before any lower respiratory illness (7–10). In addition, longitudinal cohort studies have demonstrated that infants with lower airway function before an episode of wheezing are at increased risk for subsequent episodes of wheezing in the first few years of life (11–13). The mechanisms for the lower airway function and increased frequency of wheezing associated with exposure to tobacco smoking early in life remain unclear (10). Lower airway function in infants exposed to tobacco smoking may be secondary to smaller sized airways, which could increase the risk for developing more severe airways obstruction and wheezing with lower respiratory illnesses, independent of airway reactivity. Alternatively, exposure to tobacco smoking early in life may decrease baseline airway function and increase airway reactivity with both factors being present and contributing to greater airways obstruction and wheezing with lower respiratory illnesses. The purpose of our study was to determine whether exposure to parental tobacco smoking is associated with airway hyperreactivity among healthy infants.

**METHODS**

**Subjects**

Healthy infants were recruited from general pediatric clinics within the city and from administrations. Infants were excluded from the study if they were premature at birth (< 36-weeks gestation), had congenital malformations of the cardiorespiratory system, had recurrent lower respiratory illnesses, or were hospitalized for respiratory illness. Subjects were without any respiratory symptoms for at least 3 weeks before pulmonary function testing. A history of exposure to tobacco smoking during pregnancy, postnatal environmental tobacco smoke exposure by parents, and family history of asthma and allergies was obtained at the time of testing. A positive history of asthma for mother, father, or sibling was categorized as an immediate family member, whereas a positive history for grandparent, aunt, uncle, or cousin was categorized as an extended family member. The institutional review board approved the study, and informed consent was obtained from the subjects’ parents.

**Airway Reactivity**

Infants received 50 to 75 mg/kg of chloral hydrate orally, and testing was performed while the infant was sleeping in the supine position. Baseline airway function was assessed using the raised-volume rapid thoracic compression technique as previously described (14). After completion of baseline full forced expiratory flow (FEF) volume curves, methacholine challenges (MCh) were performed as previously described (15). Two milliliters of MCh solution were placed in a nebulizer (Hudson RCI, #1882; Temecula, CA), and an aerosol was generated with 10 L/minute of compressed air, and the aerosol was combined with another source of air at 10 L/minute to produce a total flow of 20 L/minute, from which subjects inspired for 2 minutes of tidal breathing. The high flow ensured that the MCh aerosol produced by the nebulizer was contained in a total airflow that exceeded the inspiratory flow of all infants. Forced expiratory maneuvers were repeated 2 minutes after inhalation of MCh concentrations of 0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5.0, and 10 mg/ml. The challenge was stopped before the highest MCh dose if FEF75 decreased by more than 30% from baseline. After the last MCh dose, 5 mg of albuterol solution was nebulized and inhaled by the subjects.

**Tobacco Smoking Exposure**

Subjects were classified as prenatal exposure if mother smoked during pregnancy and postnatal exposure if mother or father smoked tobacco products. Hair samples were obtained from the infant at the time of evaluation. Cotinine levels (ng/mg hair) were measured by radioimmun assay at University of Toronto using a methodology previously described (16, 17).
cotinine, the log-transformed cotinine was actually used. The impact of tobacco smoking exposure on the log-transformed cotinine level was tested using an analysis of covariance. Either Pearson correlations or t tests were first used to evaluate whether age, sex, or race should be used as covariates in the model. All two-way interactions between demographics and smoke exposure were also explored to determine whether demographics influenced the impact of smoke exposure on cotinine level. A final multivariate model was run to test for significance of smoke exposure while controlling for covariates found significant by univariate analysis.

The effect of smoke exposure on baseline pulmonary function was also determined using analysis of covariance models. Pearson correlations or t tests were first used to evaluate the relationship between demographics (age, length, sex, and race) and each baseline pulmonary function test. Demographic variables, which were found to be significant, were used as covariates in the models predicting baseline pulmonary function. Similar models were used to evaluate the effect of log (cotinine) on baseline pulmonary function.

From three technically acceptable baseline curves, the mean baseline value for FEF75 was calculated. The value of FEF75 obtained after each MCh dose was expressed as the percentage change from baseline, and a dose response curve for FEF75 versus MCh was constructed. PC30 was defined as the provocative concentration of MCh that produced a 30% decrease in FEF75. If the subject did not achieve a 30% decrease after inhalation of a nebulized MCh concentration of 10 mg/ml, then the PC30 value was evaluated as 10. Infants were also characterized as either responders (>30% decrease in FEF75) or nonresponders (<30% decrease in FEF75). To determine whether the response of infants to the MCh concentration was associated with demographics and family history (history of smoke exposure, sex, race, and family history of asthma), Fisher’s exact test was used. A t test was used to test for differences in age, baseline lung function, and the log-transformed cotinine levels between infants characterized as responders and nonresponders.

Additionally, a Cox proportional hazards model was used to determine which factors affect the dose (PC30) at which infants respond. Subjects that did not respond to the drug after being given the maximum dose were censored. A separate Cox model was run for each possible explanatory variable to determine which ones to include in the final multivariate model. Variables with a p value of 0.25 or less were included in the initial full model. A manual backward stepwise analysis was then used to create a model containing only statistically significant explanatory variables of dose at time of response. Survival type curves for dose at response stratified by smoke exposure were generated using Kaplan-Meier method.

RESULTS

Subjects

There were 76 infants (33 males and 43 females) with ages that ranged between 1.5 and 34.7 months. Of the subjects 69.7% were white; 51.3% had positive history of exposure to tobacco smoking, and 34.2% had an immediate family history positive for asthma.

History of Tobacco Smoking Exposure and Hair Cotinine Level

A history of smoke exposure (no exposure, postnatal exposure, and prenatal and postnatal exposure) was found to be significantly related to hair cotinine levels using analysis of variance (p < 0.0001). Infants with history of no cigarette exposure had significantly lower levels of hair cotinine than those with history of tobacco smoking exposure either postnatal (p = 0.001) or prenatal and postnatal (p < 0.0001). In addition, those infants with prenatal and postnatal exposure had higher cotinine levels than those with only postnatal exposure, although the difference was not significant (p = 0.149). Because there was not a significant difference in cotinine level between infants with postnatal exposure only versus infants with prenatal and postnatal exposure, these two groups were combined for further analysis. While exploring possible covariates, there was no relationship found between cotinine level and either age (p = 0.561). However, there was a significant effect of race on hair cotinine levels (p < 0.0001), as well as an interaction of race and history of exposure to tobacco smoking (p = 0.031; Figure 1). Nonwhites had significantly higher levels of hair cotinine than white subjects. Also, whites with smoke exposure had significantly higher cotinine levels than whites with no smoke exposure, but there was no difference in cotinine level between nonwhites with and without smoke exposure. History of smoke exposure was still found to be significantly related to cotinine level after controlling for race (p < 0.0001).

Pulmonary Function

Both age and length were significantly correlated to all baseline lung functions; age was more strongly correlated than length, and thus, age was included in all models when testing the effect of smoke exposure on baseline lung function. FVC was significantly higher for whites than nonwhites; therefore, race was also included in the model for predicting FVC. Infants with a positive history for exposure to tobacco smoking had lower FEVs than those infants with a history negative for exposure to tobacco smoking after controlling for significant covariates; the difference was significant for FEV50,75 and FEV6 (p < 0.05) and approached significance for FEV6,5 (p = 0.059) (Table 1). There was not a statistically significant difference in FVC (p = 0.47) or FEV6 (p = 0.12) between subjects exposed to tobacco smoking and subjects not exposed. There were no significant relationships between FEVs or volumes with hair cotinine level.

Airway Reactivity

The demographics of the infants characterized as responders and nonresponders by MCh challenge are summarized in Table 2. Fourteen of the 76 subjects (18.4%) did not respond. The groups mean coefficient of variation for FEF75 calculated from the repeat baseline measurements was 8%; therefore, a 30% decrease in FEV50 was a significant decrease in airway function in response to MCh. Subjects with no tobacco smoking exposure were more likely to respond during the bronchial challenge than subjects with extrauterine only or subjects with intrauterine and postnatal smoke exposure.
TABLE 1. LEAST SQUARE MEANS (SE) OF BASELINE PULMONARY FUNCTION BY SMOKE EXPOSURE (N = 76)

<table>
<thead>
<tr>
<th>Baseline Pulmonary Function</th>
<th>No Smoke Exposure</th>
<th>Prenatal or Postnatal Smoke Exposure</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEF_{25-75}, ml/s</td>
<td>597.5 (23)</td>
<td>531.3 (22.4)</td>
<td>0.043</td>
</tr>
<tr>
<td>FEV_{0.5}, ml/s</td>
<td>303.6 (8.6)</td>
<td>280.6 (8.3)</td>
<td>0.059</td>
</tr>
<tr>
<td>FEF_{50}, ml/s</td>
<td>676.2 (24.7)</td>
<td>600.21 (24.1)</td>
<td>0.031</td>
</tr>
<tr>
<td>FEF_{75}, ml/s</td>
<td>347.1 (16.8)</td>
<td>310.1 (16.4)</td>
<td>0.120</td>
</tr>
<tr>
<td>FVC, ml</td>
<td>395.9 (13)</td>
<td>382.5 (12.6)</td>
<td>0.469</td>
</tr>
</tbody>
</table>

Definition of abbreviations: FEF = forced expiratory flow; LS = least square.

* The p value is for effect of smoke exposure on baseline pulmonary function after controlling for age. Both race and age were controlled for in the model predicting FVC.

TABLE 2. METHACHOLINE RESPONSE AT 30% REDUCTION IN FORCED EXPIRATORY FLOWS AT 75% EXPIRED VOLUME BY DEMOGRAPHICS (N = 76)

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Responders (n = 62)</th>
<th>Nonresponders (n = 14)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, weeks, mean (SD)</td>
<td>61.8 (38.7)</td>
<td>68.4 (30)</td>
<td>0.557</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>34 (79)</td>
<td>9 (21)</td>
<td>0.566</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>28 (85)</td>
<td>5 (15)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, n (%)</td>
<td>46 (87)</td>
<td>7 (13)</td>
<td>0.107</td>
</tr>
<tr>
<td>Nonwhite, n (%)</td>
<td>16 (70)</td>
<td>7 (30)</td>
<td></td>
</tr>
<tr>
<td>History of smoke exposure from parents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None, n (%)</td>
<td>35 (95)</td>
<td>2 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Extrauterine only, n (%)</td>
<td>14 (67)</td>
<td>7 (33)</td>
<td></td>
</tr>
<tr>
<td>Intrauterine and extrauterine, n (%)</td>
<td>13 (72)</td>
<td>5 (28)</td>
<td></td>
</tr>
<tr>
<td>Mother smoking while pregnant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>49 (84)</td>
<td>9 (16)</td>
<td>0.299</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>13 (72)</td>
<td>5 (28)</td>
<td></td>
</tr>
<tr>
<td>Asthma in extended family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>38 (79)</td>
<td>10 (21)</td>
<td>0.553</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>24 (86)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>Asthma in immediate family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>42 (84)</td>
<td>8 (16)</td>
<td>0.537</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>20 (77)</td>
<td>6 (23)</td>
<td></td>
</tr>
<tr>
<td>Family history of allergies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>36 (77)</td>
<td>11 (23)</td>
<td>0.225</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>26 (90)</td>
<td>3 (10)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher's Exact Test.
function. We did find that hair cotinine levels were higher in African American infants compared with white infants. This observation of a racial difference in cotinine levels is consistent with a previous report of racial differences in hair cotinine, which has been attributed to a slower metabolism of nicotine to cotinine in African Americans than whites (19–21). The absence of a relationship between airway function and hair cotinine in our study may reflect limitations in the use of hair cotinine as a quantitative measure of chronic exposure to tobacco smoking or that the effect of tobacco smoking exposure on airway function is not directly related to cotinine but on other products of tobacco smoke exposure that were not measured.

Although our infants with a history of exposure to tobacco smoking had lower baseline airway function, they were less reactive to inhaled MCh than infants with a history negative for tobacco exposure. This finding is consistent with the recent report by Joad and colleagues who found that combined prenatal and postnatal exposure of monkeys to environmental tobacco smoke was associated with lower, not higher, airway reactivity to inhaled MCh when assessed using partial forced expiratory maneuvers (23). Young and coworkers, who assessed airway responsiveness to inhaled histamine in healthy infants using partial flow volume maneuvers (24), reported that parental smoking was associated with heightened airway reactivity; however, in a subsequent report the investigators found that airway reactivity among healthy infants was not related to parental tobacco smoking (25). Cumulatively, the findings from these studies suggest that exposure to tobacco smoking early in life is not associated with heightened airway reactivity early during infancy. We also found that among healthy infants a positive family history of asthma or allergy was associated with heighten airway reactivity, independent of exposure to tobacco smoking. This finding related to family history of asthma/allergy is consistent with the study by Young and coworkers (24).

Although exposure to tobacco smoking is associated with lower baseline lung function, as well as an increased risk of wheezy lower respiratory illnesses, it is unclear whether heightened airway reactivity is a mechanism that contributes to recurrent wheezing early in life. After bronchiolitis, several studies have reported lower airway function; however, studies have differed as to whether there was also heightened airway reactivity (23, 26–28). A potential mechanism for lower airway function in infants exposed to parental tobacco smoking include airways that are absolutely smaller in caliber relative to somatic size, thickened airway walls, more compliant airway walls, increased airway smooth muscle tone, decreased pulmonary elastic recoil, as well as airways inflammation. All of these mechanisms could potentially contribute to heightened airway reactivity; however, thickened airway walls that are also stiffer could produce a decrease in airway caliber and a decrease in airway reactivity (29, 30). An autopsy study of infants that died from sudden infant death syndrome found thicker airway walls in those infants with a history of maternal smoking compared with infants with history negative for maternal smoking (31). In addition, prenatal nicotine exposure of rhesus monkeys produced a decrease in airway function and increased collagen expression in the airway walls (32, 33). Therefore, thickened and stiffer airway walls are potential mechanisms that could increase the risk of recurrent episodes of wheezing early in life but not produce an increase in airway reactivity. Exposure to tobacco smoking early in life may not contribute to the development of airway hyperreactivity, but rather, exposure to tobacco smoking may be an important factor that contributes to lower airway function and recurrent airways obstruction in infants with “transient” wheezing, as well as an important trigger factor for increased respiratory symptoms in subjects with airway hyperreactivity. Prenatal and postnatal

### Table 3. Univariate Results of Censored Regression of DOSE at Response of PC<sub>30</sub>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoke exposure from parents (yes vs. no)</td>
<td>0.42</td>
<td>(0.25, 0.70)</td>
<td>0.001</td>
</tr>
<tr>
<td>Log Cotinine</td>
<td>0.90</td>
<td>(0.77, 1.04)</td>
<td>0.164</td>
</tr>
<tr>
<td>Age, weeks</td>
<td>1.00</td>
<td>(0.99, 1.00)</td>
<td>0.351</td>
</tr>
<tr>
<td>Sex, male vs. female</td>
<td>1.36</td>
<td>(0.82, 2.25)</td>
<td>0.234</td>
</tr>
<tr>
<td>Race, white vs. nonwhite</td>
<td>1.68</td>
<td>(0.95, 2.99)</td>
<td>0.066</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;75&lt;/sub&gt;, Z score</td>
<td>1.06</td>
<td>(0.84, 1.34)</td>
<td>0.627</td>
</tr>
<tr>
<td>Asthma in extended family</td>
<td>1.66</td>
<td>(0.99, 2.79)</td>
<td>0.060</td>
</tr>
<tr>
<td>Asthma in immediate family</td>
<td>0.86</td>
<td>(0.51, 1.47)</td>
<td>0.583</td>
</tr>
<tr>
<td>Family history of allergies</td>
<td>1.62</td>
<td>(0.97, 2.70)</td>
<td>0.067</td>
</tr>
</tbody>
</table>

* Definition of abbreviation: FEF = forced expiratory flow; PC<sub>30</sub> = the provocative concentration of methacholine challenges that produced a 30% decrease in FEF<sub>75</sub>.

* Likelihood ratio test.

† None vs. postnatal smoke exposure, p = 0.014. None vs. prenatal and postnatal smoke exposure, p = 0.004. Postnatal vs. prenatal and postnatal smoke exposure, p = 0.683.
exposure to tobacco smoking could produce different effects upon pulmonary function and airway reactivity; however, the number of infants evaluated in our study and the high concordance between prenatal and postnatal exposure to tobacco smoking precluded a separate analysis.

In summary, we have found that parental tobacco smoking was associated with lower baseline airway function and less reactive airways among healthy infants. In addition, a family history of asthma/allergies was associated with greater airway reactivity. We speculate that heightened airway reactivity is not the mechanisms accounting for transient wheezing early in life; however, heightened airway reactivity associated with family asthma/allergy may be present in infants that will develop persistent wheezing into childhood or late onset wheezing.

Conflict of Interest Statement: R.S.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; T.W.-N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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