IMPORTANCE  Both maternal smoking during pregnancy and low birth weight have been implicated in impaired development of the retina.

OBJECTIVE  To investigate the associations of maternal smoking during pregnancy and low birth weight with retinal nerve fiber layer (RNFL) thickness in preadolescent children.

DESIGN, SETTING, PARTICIPANTS  The Copenhagen Child Cohort 2000 Eye Study is a prospective, population-based, birth cohort study that included all children (n = 6090) born in 2000 in Copenhagen, Denmark. Maternal smoking data were collected through parental interviews. Birth weight, pregnancy, and medical history data were obtained from the Danish Medical Birth Registry. As a follow-up, the study performed eye examinations on 1406 of these children from May 1, 2011, to October 31, 2012, when the children were aged 11 or 12 years. The participants were predominantly (1296 [92.4%]) of European descent. Study data were analyzed from June 1, 2016, to October 1, 2016.

MAIN OUTCOMES AND MEASURES  Peripapillary RNFL thickness measured using optical coherence tomography at the 11- or 12-year examination.

RESULTS  Of the 1406 children in the study, 1323 were included in the analysis (mean [SD] age was 11.7 [0.4] years; 633 [47.8%] were boys and 690 [52.2%] were girls). The mean (SD) RNFL thickness was 104 (9.6) μm. In 227 children whose mothers had smoked during pregnancy, the peripapillary RNFL was 5.7 μm (95% CI, 4.3-7.1 μm; P < .001) thinner than in children whose mothers had not smoked after correction for age, sex, birth weight, height, body weight, Tanner stage of pubertal development, axial length, and spherical equivalent refractive error. In low-birth-weight children (<2500 g), the RNFL was 3.5 μm (95% CI, 0.6-6.3 μm; P = .02) thinner than in normal-birth-weight children after adjustment for all variables.

CONCLUSIONS AND RELEVANCE  Exposure to maternal smoking during uterine life and low birth weight were independently associated with having a thinner RNFL at age 11 or 12 years. These observations support previous findings that intrauterine and perinatal factors can have long-lasting effects on the retina and the optic nerve. The results of this study add evidence to existing recommendations to avoid smoking during pregnancy and support measures that promote maternal and fetal health.
Fetal development has a considerable effect on the central nervous system, including the retina and the optic nerve.\textsuperscript{1-4} Toxic effects on the fetus of maternal tobacco consumption have been observed, and smoking is associated with adverse birth events.\textsuperscript{5} Understanding these adverse consequences is important because as many as 15\% to 30\% of women in developed countries smoke tobacco during pregnancy.\textsuperscript{6,7}

Several studies have shown an association between maternal smoking during pregnancy and intrauterine growth restriction.\textsuperscript{5,8,10} Because low birth weight has been associated with having a thinner retinal nerve fiber layer (RNFL) later in life,\textsuperscript{11,12} smoking could have both direct and indirect effects on the optic nerve and its connections in the retina. A study in Spain of 70 children attending hospital clinics for various conditions found an association between maternal smoking and the children’s thinner RNFL, regardless of the birth weight.\textsuperscript{3} Because such findings are of vital importance for lifestyle counseling, we used the Copenhagen Child Cohort 2000 (CCC2000) Eye Study to validate the results of the Spanish study.

This prospective, population-based study examined a large birth cohort of Danish children to assess the relative effects of maternal smoking and birth weight on the status of the RNFL in late childhood (ages 11 or 12 years).

Methods

The CCC2000 study comprised all children (n = 6090) born in 16 municipal districts in greater Copenhagen, Denmark, in 2000 and conducted follow-up eye examinations of 1406 children between May 1, 2011, and October 31, 2012, when the children were aged 11 or 12 years.\textsuperscript{13} Information about pregnancy and early medical history was obtained from the CCC2000 study activities and from the Danish Medical Birth Registry. Medical history was supplemented by interviews with the parents during the follow-up examination. This study was approved by the Health Research Ethics Committee in Region Hovedstaden, Denmark, and was performed in accordance with the Helsinki Declaration.\textsuperscript{14} Informed written consent was obtained from the children’s parents or legal guardians prior to the examinations.

This present analysis excluded 83 children who had prior eye trauma, congenital malformations, corrected visual acuity poorer than 80 Early Treatment Diabetic Retinopathy Study (ETDRS) letters at 4 m distance (Snellen 20/25), an inability to cooperate with axial length measurement, an inability to obtain optical coherence tomography scans of acceptable quality, or an inability to cooperate with other aspects of the eye examination. Thus, the final analysis included only 1323 children of the 1406 participants in the follow-up examination. Only right eyes were included in the analysis. According to the parents, none of the children had been diagnosed with retinopathy of prematurity or treated during infancy for eye disease other than conjunctivitis.

Noncycloplegic objective refraction obtained by an automated refractometer (Retinomax K-Plus 2; Righton) was used to guide subjective refractioning, which was performed by adding positive power (+0.5 diopters) consecutively to the objective refraction until a loss of at least 3 letters was observed and then by removing positive lens power until maximum visual acuity was achieved again. Pharmacological cycloplegia was avoided because ascertainment might be compromised if eye drops were used, especially among the mentally vulnerable children, who were of particular interest to the core pediatric psychiatry component of the CCC2000 study.

Ocular axial length was measured using an interferometric device (IOLMaster, version 3.01.0294; Carl Zeiss Meditec). Uncorrected and best-corrected visual acuity were determined using ETDRS charts and a refraction protocol, which has been described in a previous article.\textsuperscript{13}

Information about self-reported maternal smoking during pregnancy and birth weight was retrieved from the Danish Medical Birth Registry, a record of obligatory reports about pregnancy, birth, and infancy health characteristics submitted by midwives and obstetricians in Denmark. Pregnant women in the country were routinely offered 3 physician consultations and 4 to 6 midwife consultations during pregnancy.\textsuperscript{15}

Information about the citizenship of the mother was pulled from the Danish Civil Registration System. Citizenship codes were grouped into 5 categories based on the dominating racial/ethnic descent in the country: European, Asian, African, Middle Eastern, and other or unknown.

Maternal smoking was categorized as (1) nonsmoking from before conception, (2) smoking at conception but stopped during pregnancy, and (3) continued smoking throughout pregnancy. No data were available on the number of cigarettes smoked daily during the pregnancy or on the number of pack-years smoked by the mother before and after pregnancy. No data were available on paternal smoking history or on maternal alcohol consumption during or before pregnancy. Birth weight (available for 1284 children) was categorized as low (<2500 g), normal (2500-4500 g), and high (>4500 g).

We measured RNFL thickness using optical coherence tomography (Spectralis HRA+OCT; Heidelberg Engineering). Scanning procedures included a standard 12\°, disc-centered circular peripapillary scan in high resolution, averaging more than 25 B-scans. We did not use the manufacturer’s method\textsuperscript{16} of correcting for lateral magnification using corneal curvature data.

### Key Points

**Question** What is the association of maternal smoking during pregnancy and low birth weight with retinal nerve fiber layer thickness later in the child’s life?

**Findings** This population-based study of 1406 children in Denmark found that children’s in utero exposure to maternal smoking and low birth weight were independently associated with the thinner retinal nerve fiber layer observed during these children’s preadolescent years.

**Meaning** These findings add evidence to existing recommendations for expectant mothers to avoid smoking during pregnancy and add support to other measures that promote maternal and fetal health.
Scaling for differences in lateral magnification between eyes helps obtain better measurements of lateral distances, thus enabling the circular scan to be placed at uniform distances from the center or the margin of the disc. We adjusted, however, for axial length and refraction, 2 key determinants of lateral magnification, in the statistical analysis. We measured RNFL thickness automatically using the manufacturer’s software (Heidelberg Eye Explorer, version 1.6.1.0; Heidelberg Engineering), and we reported the measurements as mean circumpapillary RNFL thickness and sectorial peripapillary RNFL thickness in these 6 sectors: temporal, nasal, superotemporal, inferotemporal, superonasal, and inferonasal. We manually centered each optic disc scan during scan acquisition, and we repeated or excluded RNFL scans with a quality less than 25 dB or with an unclear RNFL border. We did not adjust for fovea-to-Bruch’s membrane opening axis deviation.

At the 11- or 12-year follow-up eye examinations, height without shoes, body weight, and blood pressure were obtained for each child. Self-reported pubertal development was assessed by showing the children gender-specific drawings illustrating the Tanner puberty stages 1 through 4 and then asking the children to choose the drawing that best represented their stage of pubertal development.\(^{17}\)

Statistical analyses were performed from June 1, 2016, to December 1, 2016 using SAS statistical software, version 9.3 (SAS Institute Inc). Means and SDs were calculated for continuous variables; medians and interquartile ranges were calculated for skewed distributions (eg, body weight). Comparison between groups defined by smoking status was made using 1-way analysis of variance or nonparametric Wilcoxon signed rank test (eg, body weight) or using \(\chi^2\) or Fisher exact tests (eg, Tanner stages and birth weight groups). Associations between RNFL thickness and other variables were assessed using a general linear model. Maternal smoking status, Tanner stage, and sex were entered as categorical data. Birth weight, height, body weight, axial length, spherical equivalent refractive error, and age were entered as continuous data. Age was calculated in days, but results are presented in years. Spherical equivalent refraction was calculated as the algebraic sum of the value of the sphere and half the cylinder. The assumptions of linearity, variance homogeneity, and normality of the distribution of residuals underlying the statistical model were assessed by review of relevant plots. The analyses were adjusted by including all variables in the general linear model, and estimates are presented with 95% CIs. The level of significance was set at 2-sided \(P < .05\).

### Results

A total of 1332 children were included in the present study (mean [SD] age, 11.7 [0.4] years; 633 (47.8%) were boys and 690 (52.2%) were girls; 1296 (92.4%) of European descent). The mean (SD) peripapillary RNFL thickness was 104 (9.6) \(\mu\)m. Data for maternal smoking status during pregnancy were available for 1289 of the children, who were predominantly of European descent (Table 1). The mothers of 1035 children (80.3%) did not smoke during pregnancy, and the mothers of 227 (17.6%) continued smoking during pregnancy, and the mothers of 227 (17.6%) continued smoking throughout pregnancy (Table 1). Birth weight was available for 1284 children, of whom 50 (3.9%) were of low birth weight and 47 (3.7%) were of high birth weight. Of the low-birth-weight group, 1 child was of extremely low birth weight (<1000 g) and 1 was of very low birth weight (1000 to <1500 g). The mean (SD) birth weight of children whose mothers had smoked was 3357 (636) g, which was lower than the 3578 (569) g found in children whose mothers had not smoked (Table 1).

Children whose mothers did not smoke during pregnancy had a mean RNFL thickness of 105 (9.4) \(\mu\)m (Table 1). Children whose mothers smoked throughout pregnancy had a thinner peripapillary RNFL than the children of nonsmoking mothers (−5.2 \(\mu\)m; 95% CI, −6.6 to −3.8 \(\mu\)m; \(P < .001\) [crude analysis]) (Table 2). The smoking-related deficit remained significant and of comparable sign and magnitude (−5.7 \(\mu\)m; 95% CI, −7.1 to −4.3 \(\mu\)m; \(P < .001\); Table 2) after adjusting for birth weight, age, sex, body weight, Tanner stage, axial length, and spherical equivalent refractive error. Subdivision into peripapillary sectors showed that the effect of smoking was found in all 6 sectors and was of the highest magnitude in the inferonasal (−9.7 \(\mu\)m; 95% CI, −13.1 to −6.3 \(\mu\)m; \(P < .001\)) and inferotemporal (−8.9 \(\mu\)m; 95% CI, −11.7 to −6.1 \(\mu\)m; \(P < .001\)) sectors (Table 3). There was no difference in RNFL thickness when comparing children of mothers who ceased smoking during pregnancy with children of nonsmoking mothers (Table 2).

Compared with children of normal birth weight, those with low birth weight had a thinner RNFL, the deficit being −4.7 \(\mu\)m (95% CI, −7.5 to −2.0 \(\mu\)m; \(P = .001\)) in the crude analysis and −3.5 \(\mu\)m (95% CI, −6.3 to −0.6 \(\mu\)m; \(P = .02\)) after multiple adjustments (Table 2). In an analysis of birth weight as a continuous variable, RNFL thickness increased by 0.22 \(\mu\)m (95% CI, 0.13–0.31 \(\mu\)m; \(P = .001\) [crude analysis]) per 1-g increase in birth weight (Table 2). The association between birth weight and RNFL thickness remained significant in the multivariate analysis at 0.17 \(\mu\)m (95% CI, 0.08–0.26 \(\mu\)m; \(P = .004\)) per 1-g increase in birth weight (Table 2). In the sectorial analysis, the largest adjusted deficits in children of low birth weight occurred in the nasal and inferonasal sectors (Table 3).

A longer axial length was associated with a thinner RNFL (−3.0 \(\mu\)m/mm; 95% CI, −3.7 to −2.3 \(\mu\)m/mm; \(P < .001\)), as was a more myopic refraction in the crude analysis (Table 2). In the multivariate analysis, higher body height was significantly associated with an increasing RNFL thickness (0.11 \(\mu\)m/cm; 95% CI, 0.02–0.21 \(\mu\)m/cm; \(P = .02\)), and girls had a thinner RNFL than boys did (−1.7 \(\mu\)m; 95% CI, −2.8 to −0.5 \(\mu\)m; \(P = .004\)) (Table 2). The associations with axial length and refractive error persisted in the multivariate analysis. Retinal nerve fiber layer thickness was not associated with age, pubertal development, or body weight in crude or multivariate analyses.

### Discussion

This study found that both maternal smoking during pregnancy and low birth weight were independently associated with a thinner peripapillary RNFL at age 11 or 12 years. In
addition, longer axial length, a more myopic refractive error, female sex, and shorter stature were associated with a thinner RNFL.

To our knowledge, the effect of maternal smoking and birth weight on RNFL status later in life has not previously been examined in a large population-based cohort. Our finding in a co-
Association of Maternal Smoking and Birth Weight With Later RNFL Thickness

Table 3. Sectorial Peripapillary RNFL Thickness in Children Aged 11 or 12 Years According to Maternal Smoking Status During Pregnancy and Birth Weight

<table>
<thead>
<tr>
<th>Factor</th>
<th>RNFL Thickness by Sector, Mean (SD)*</th>
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<tbody>
<tr>
<td></td>
<td>Temporal</td>
</tr>
<tr>
<td>Maternal smoking status during pregnancy</td>
<td></td>
</tr>
<tr>
<td>Did not smoke (n = 1008)</td>
<td>74.4 (10.2)</td>
</tr>
<tr>
<td>Ceased smoking (n = 27)</td>
<td>73.7 (11.0)</td>
</tr>
<tr>
<td>Continued smoking (n = 221)</td>
<td>70.6 (9.1)</td>
</tr>
<tr>
<td>Continued smoking vs did not smoke, adjusted mean difference (95% CI)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Birth weight groups, mean (SD)*</td>
<td></td>
</tr>
<tr>
<td>Low, &lt;2500 g (n = 47)</td>
<td>71.4 (8.9)</td>
</tr>
<tr>
<td>Normal, 2500-4500 g (n = 1160)</td>
<td>73.9 (10.3)</td>
</tr>
<tr>
<td>High, &gt;4500 g (n = 44)</td>
<td>75.2 (8.6)</td>
</tr>
<tr>
<td>Low birth weight vs normal birth weight, adjusted mean difference (95% CI)</td>
<td>.45</td>
</tr>
</tbody>
</table>

* Data are presented as unadjusted means (SDs) unless otherwise indicated.

* Adjusted for age, sex, height, weight, Tanner stage, axial length, spherical equivalent refractive error, birth weight, and maternal smoking during pregnancy.

The strengths of the present study include continuous data collection since early pregnancy, the use of high-resolution optical coherence tomography, and the availability of data for pubertal development and axial length. The reliability of self-reported maternal tobacco consumption is supported by an earlier study that compared this source of information with plasma and/or urine concentrations of cotinine, the primary metabolite of nicotine.18

The 17.6% of mothers who smoked during pregnancy is comparable to the 11.2% found in another study from Denmark, conducted from 2003 to 2006.19 The birth weight distribution in our study was representative of the general birth weight distribution in Denmark in 2000, when 3.4% of children were of low birth weight and 4.3% were of high birth weight.20

Maternal smoking has been shown to be associated with subnormal cerebral cortex thickness21-23 and is likely to have deleterious consequences on cerebral function.24 Animal studies have shown that prenatal exposure to nicotine alone caused structural changes in the cerebral cortex that are likely to be associated with cognitive dysfunction.25,26 Thus, it is not surprising that maternal smoking was found to be associated with a thinner RNFL in late childhood. It remains to be analyzed whether RNFL characteristics are associated with intellectual function in the CCC2000 study, and only long-term follow-up can determine whether there is an association between thin RNFLs and risk of eye diseases, such as glaucoma, in adulthood.

Of note, the RNFL deficits found in this study were most prominent in the inferonasal sector of the optic disc, where such defects are also most pronounced in OPA1 (OMIM 165500) autosomal dominant optic atrophy, which is caused by mitochondrial dysfunction.27

The number of mothers in this study who gave up smoking after they had learned they were pregnant was small (n = 27). The lack of a demonstrable RNFL deficit in their children may be a spurious finding, but no evidence suggests that it can ever be too late to stop smoking during pregnancy.

Our findings complement the results obtained by Tariq et al11 and support, with a newer optical coherence tomographic technique and with correction for smoking, that a higher birth weight is associated with a higher mass of retinal nerve fiber tissue later in life. It remains to be determined whether more tissue means a higher number of optic nerve...
fibers and better resistance to optic nerve diseases such as glaucoma,28 in which a large retinal nerve fiber reserve appears to protect against the gradual development of visual field defects.

The attenuation of the RNFL with increasing ocular axial length and increasingly myopic refraction may be a simple effect of eyeball elongation and distention of the retina.29-33 but the association of glaucoma with myopia suggests that this attenuation is not without adverse consequences.

After multiple adjustments, but not in the crude analysis, we found that girls had a 1.7-μm thinner RNFL than boys did. There is no consistent pattern in previous studies, which should motivate a meta-analysis when a substantial amount of data obtained with up-to-date methods are available.33-35

Not only does low birth weight predispose individuals to hypertension and cardiovascular disease,36 but we found it is also associated with a thinner RNFL. This finding adds to the complexity of the search for risk factors for suboptimal development of the eye given that birth weight can be influenced by a multitude of environmental factors and can be affected by a large number of loci in the human genome.36

**Limitations**

A limitation of the data analysis is that it does not include socioeconomic status, which is likely to be associated with tobacco consumption in the CCC2000 study as in other studies.37 Consequently, there may be residual, unresolved confounding from socioeconomic effects included in the association with smoking. Of importance, we did not have information on maternal alcohol consumption during pregnancy, which is a potential major confounder because alcohol is linked with smoking38 and affects fetal development.39 In addition, exposure to tobacco smoke in the form of passive smoking is likely to have continued after birth in children exposed to tobacco during pregnancy, and we do not have information on this factor or on paternal smoking habits. We could not test for any dose-response effect of smoking during pregnancy because we did not have data on the number of cigarettes smoked per day or the number of pack-years smoked before and after the pregnancy. We did not register race/ethnicity specifically, but the citizenship of the mother was available from the Danish Civil Registration System.

Another limitation of the study is that the effects of variations in lateral magnification of the confocal scanning laser component of the Spectralis HRA+OCT were not accounted for by entering corneal curvature data before the scan but instead by statistical adjustments for axial length and refraction in the data analysis.

Compared with the rest of the cohort, the 1406 participants in the follow-up examination often had 2 Danish parents, more well-educated parents, and more stable families with higher annual household income.17 Hence, the study population does not fully represent the general population of Copenhagen. However, no substantial differences were seen regarding Apгар score and birth weight between the children included in the study and all Danish children born in 2000.

**Conclusions**

Exposure to maternal smoking during uterine life and low birth weight were independently associated with having a thinner RNFL at age 11 or 12 years in Danish children. These observations support hypotheses that intrauterine exposures can have long-lasting effects on the structure of the retina and the optic nerve. Our findings add evidence to existing recommendations against smoking and support measures that promote maternal and fetal health.
Evaluation of Retinal Nerve Fiber Layer Thinning With Fourier-Domain Optical Coherence Tomography

Christopher Kai-Shun Leung, MD

Retinal nerve fiber layer (RNFL) defects are a defining feature of optic neuropathies and have been implicated in a few neurodegenerative disorders, including multiple sclerosis, Alzheimer disease, and Parkinson disease. Ashina et al conducted a prospective study observing 1406 children recruited from the Copenhagen Child Cohort over 10 years. They showed that children of mothers with a history of smoking during pregnancy had a mean reduction in average circumpapillary RNFL thickness of 5.7 μm, adjusting for axial length, birth weight, and other covariates, compared with children without such history. Smoking during pregnancy has a detrimental effect on fetal development, including a higher risk of birth defects, low birth weight, and development of cognitive and behavioral problems. Although a difference of 5 to 6 μm in average circumpapillary RNFL thickness is unlikely to translate into a detectable difference in visual function in children aged 12 to 13 years, the risk of subsequent development of visual impairment should not be overlooked.

In the 1958 British birth cohort study observing 9330 participants for more than 40 years, adults aged 44 to 45 years with a history of maternal smoking during pregnancy had an odds ratio of 1.35 (95% CI, 1.01-1.82) for impaired near vision.