Smoking Cessation Early in Pregnancy and Birth Weight, Length, Head Circumference, and Endothelial Nitric Oxide Synthase Activity in Umbilical and Chorionic Vessels

An Observational Study of Healthy Singleton Pregnancies

Malene R. Andersen, MSc, PhD; Ulf Simonsen, MD, PhD; Niels Uldbjerg, MD, PhD; Christian Aalkjær, MD, PhD; Steen Stender, MD, PhD

Background—Reduced production of the vasodilator nitric oxide (NO) in fetal vessels in pregnant smokers may lower the blood flow to the fetus and result in lower birth weight, length, and head circumference. The present study measured endothelial NO synthase (eNOS) activity in fetal umbilical and chorionic vessels from nonsmokers, smokers, and ex-smokers and related the findings to the fetal outcome.

Methods and Results—Of 266 healthy, singleton pregnancies, 182 women were nonsmokers, 43 were smokers, and 41 stopped smoking early in pregnancy. eNOS activity and concentration were quantified in endothelial cells of the fetal vessels. Cotinine, lipid profiles, estradiol, L-arginine, and dimethylarginines that may affect NO production were determined in maternal and fetal blood. Serum cotinine verified self-reported smoking. Newborns of smokers had a lower weight (P < 0.001) and a smaller head circumference (P < 0.041) and were shorter (P < 0.001) than newborns of nonsmokers and ex-smokers. eNOS activity in umbilical veins of smokers was 36% lower (P < 0.001), eNOS concentration was 47% lower (P < 0.001), and the fetal plasma level of high-density lipoprotein was 18% lower (P < 0.001) than those of nonsmokers, whereas the same levels were found in umbilical veins from ex-smokers and nonsmokers. The same patterns in eNOS activity and concentration were found in umbilical arteries and chorionic vessels. Fetal plasma levels of estradiol, L-arginine, dimethylarginines, total cholesterol, and triglycerides were similar for nonsmokers, smokers, and ex-smokers.

Conclusions—The findings suggest that maternal smoking reduces eNOS activity in the fetal vascular bed, contributing to retarded fetal growth caused by the reduction of vasodilatory capacity, and suggest that smoking cessation early in pregnancy prevents these effects in newborns. (Circulation. 2009;119:857-864.)

Key Words: endothelium ■ nitric oxide synthase ■ pregnancy ■ smoking ■ vessels ■ lipids

Maternal smokers deliver infants with lower birth weight than nonsmokers.1 Birth weight decreases with increasing number of cigarettes smoked per day after the fourth month of pregnancy, and cessation of smoking before the fourth month yields birth weights comparable to those of infants born to nonsmokers.2,3 The lower birth weight of fetuses exposed to maternal smoking may be due to a shorter gestational age4,5 or a slower fetal growth.6

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A mechanism by which smoking causes lower birth weight is the well-documented chronic7,8 and acute9-11 enhanced blood flow velocity in different fetal vascular beds, indicating a greater vascular resistance. Likewise, in uterine arteries, enhanced blood flow velocity indicates a greater resistance in maternal smokers.7,8 If increased resistance followed by reduced flow despite increased blood flow velocity affects the vessels taking oxygen and nutrients to the fetus, it is understandable that fetal growth in smoking mothers will be restricted. A number of studies in nonpregnant women concerning endothelium-dependent vasodilatation suggest that the reduced production of the vasodilator nitric oxide (NO) is instrumental for the reduced flow caused by smoking.12-14 NO is produced by the endothelial intracellular enzyme endothelial NO synthase (eNOS) when L-arginine is converted into L-citrulline. Because NO also involves other homeostatic functions in the fetus, impaired vasodilation may not be the only mechanism restricting fetal growth in smokers.
Several studies suggest that NO deficiency is related to intrauterine (fetal) growth restriction. For instance, women with intrauterine growth–restricted pregnancies have a lower NO-dependent flow-mediated dilation and a higher plasma level of asymmetric dimethylarginine (ADMA), an inhibitor of eNOS that competes with l-arginine, than women with normal pregnancies. In addition, a lower eNOS expression in the umbilical artery and a lower eNOS activity in placental villus tissue are found in intrauterine growth–restricted pregnancies compared with normal pregnancies. In an earlier study, we found a smaller fetal outcome and lower eNOS activity and concentration in fetal umbilical veins exposed to maternal smoking compared with those of lifelong nonsmoking mothers.

After smoking cessation, it took ≈6 years to increase the endothelium-dependent vasodilatation by ≈50% in healthy young adults, suggesting that endothelial cells (ECs) in ex-smokers only very slowly regain a nonsmoking NO production. The present study was designed to compare the endothelial function in fetal vessels measured by the eNOS activity in uncultured ECs obtained from umbilical and chorionic vessels with the pregnancy outcomes of nonsmokers, smokers, and ex-smokers. The smoking status was validated by serum cotinine.

Methods

Study Participants

Some 756 randomly selected women with singleton pregnancies admitted to the Department of Obstetrics and Gynecology (June 2003 to April 2004) received written descriptions of the nature and purpose of the study without our knowledge of their smoking habits. Women with diabetes mellitus, multiple gestations, previously pregnant women with stillbirth, intrauterine growth restriction, or congenital malformation were not included in the study. Although women with stillbirth, intrauterine growth restriction, or congenital malformations were not invited to participate in the study. At the antenatal visit at ≤12 weeks’ gestation, the women were informed verbally about the study. Written consent was obtained from 557 of the invited women. The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee (1988/1349, 1991/2060, 20010077, and 20030274) and the Danish Data Surveillance Authority (2004–41–4001).

Women were excluded from the study if they had cardiovascular diseases before pregnancy (n=15), gestational diabetes (n=3), or preclampsia (n=4) or if they delivered before 27 completed weeks of gestation (n=42). In addition, women were omitted from the study because of spontaneous or induced abortion or fetal death or because they delivered at home or at another hospital (n=52). Although samples were collected 24 hours a day, 175 of the women gave birth to term deliveries were obtained from a birth registration form filled in by the delivery was obtained from a questionnaire (n=262). Information about the delivery was obtained from a birth registration form filled in by the attending midwife immediately after delivery.

Caffeine intake per day was calculated from the intake of coffee (1 cup=100 mg), tea, chocolate (1 cup=50 mg), and cola (a 0.25-L bottle=50 mg). Cotinine and estradiol were quantified in maternal serum obtained at ≈12, ≈20, and ≈39 weeks of gestation and fetal cord serum by chemiluminescent immunoassays (EURO/DPC Ltd, DPC Scandinavia, Mölndal, Denmark) for the IMMULITE 2500 analyzer. The detection limit of cotinine is 10.0 ng/mL.

Determination of Lipid Profiles

Total cholesterol, high-density lipoprotein (HDL), and triglyceride levels were quantified in nonfasting maternal plasma obtained at ≈20 weeks of gestation and fetal cord plasma by routine enzymatic assays (Roche Diagnostics, Hvidovre, Denmark) for the COBAS Integra 400 analyzer. Very-low-density lipoprotein (VLDL) was calculated from the amount of triglycerides multiplied by 0.45 and low-density lipoprotein (LDL) from the amount of total cholesterol minus the amount of HDL and VLDL. Calculations were not performed on fetal plasma values because of limitations of Friedwald’s formula.

Determination of l-Arginine and Dimethylarginines

l-Arginine, N⁵,N⁷-dimethyl-l-arginine (ADMA), and N⁵,N⁷-dimethyl-l-arginine (symmetric dimethylarginine) were quantified in maternal plasma obtained at ≈39 weeks of gestation and fetal cord plasma by high-performance liquid chromatography (fluorescence detector) with precolumn derivation of o-phthalaldehyde, using l-homoarginine as internal standard according to previous descriptions.

Preparation of ECs

Immediately after delivery of the placenta, fetal venous cord blood was collected, and the placenta, including the cord, was weighed. Umbilical and chorionic ECs were prepared according to previous descriptions. In short, the cord segment (≈10 cm) adjoining the placenta and chorionic vessels was removed, rinsed with 0.9% saline on ice, and divided into veins and arteries. The vessels were opened longitudinally, and the ECs were isolated by a single scrape of the luminal surface with a razor blade. The EC samples were frozen and stored at −80°C until assayed. The samples were analyzed at the end of the study without knowledge of the mothers’ smoking habits. The number of ECs in the sample was determined by duplicate cell counting in a Burker-Türk counting chamber at the light microscopic level.

Citrulline Assay

The eNOS activity in the samples was quantified by duplicate determination of the conversion of [14C]-L-arginine to [14C]-L-citrulline with a few modifications of the methods previously described. Briefly, ECs were homogenized by 6 cycles of freeze-thawing, and cell homogenate was diluted 1:6 with a Tris-reaction buffer containing [14C]-L-arginine, calcium, and the cofactors: calmodulin, tetrahydrobiopterin, flavin adenine dinucleotide, and β-nicotinamide adenine dinucleotide phosphate (reduced form), was incubated for 30 minutes at 37°C. [14C]-L-citrulline was isolated by column chromatography and quantified by liquid scintillation counting. Finally, eNOS activity was calculated from the mean formation of [14C]-L-citrulline per minute and the mean concentration of ECs in the sample (picomoles L-citrulline per minute per 10⁶ ECs).

Previously, it was reported that in control samples the eNOS activity did not change during the first 18 months of storage at −80°C. In the present study, EC samples were stored for 5 to 22 months until assayed. eNOS activity was not affected by increasing periods of storage within that time frame (r=−0.04, P=0.5).

For internal quality assurance, ECs from pig aortas (Danish Crown, Odense, Denmark) that had an eNOS activity of 34.8±5.7 pmol L-citrulline per minute per 10⁶ ECs (mean±SD) were used for determination of within-series and between-series coefficients of variation (CVs). The within-series CV was 6% (n=6) and the between-series CV was 11% (n=71) when measured within 12 months. For human ECs (pool of cells from umbilical and chorionic
was performed, and the partial correlation coefficient, 

\[ r = 0.10, \ P = 0.1 \]

For internal quality assurance, controls of recombinant human eNOS standards were used. The within-series CVs were 5% for a low control (mean, 0.1 ng/mL; n = 6) and 3% for a high control (mean, 3.8 ng/mL; n = 6). Between-series CVs were 2% to 5% (n = 37) for concentrations within the mean range, 0.1 to 3.8 ng/mL, when measured within 8 months. For human ECs (pool of cells from umbilical and chorionic vessels; mean, 1.7 ng eNOS/10^6 ECs), the eNOS concentration was not affected by increasing periods of storage within that time frame (\( r = 0.10, \ P = 0.1 \)).

For the examination of the association between 2 variables after adjustment for the effect of other variables, multiple linear regression analysis was performed, and the partial correlation coefficient, \( r \), was estimated. Only for multiple linear regression analysis were the fetal plasma levels of HDL, eNOS activity, and eNOS concentration logarithmically transformed because these data were highly skewed. Statistical calculations were carried out with the Stata 8.2 computer program (Stata Corp, College Station, Tex); differences were considered significant at \( P < 0.001 \).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Clinical, Sociodemographic, and Lifestyle Characteristics**

Table 1 shows that maternal nonsmokers, smokers, and ex-smokers were similar in age, body mass index before pregnancy, blood pressure, and parity but were not comparable with respect to marital status, years of schooling, and occupational status. The nonsmokers and ex-smokers were more likely to be cohabiting and had a significantly higher level of education than the smokers. In addition, the percentage of students among nonsmokers was higher than among smokers, and the percentage of unemployed was higher among smokers than in the other 2 groups.

The self-reported daily caffeine intake was \( \approx 1.9 \)- and \( \approx 1.5 \)-fold higher among smokers than among nonsmokers and ex-smokers, respectively. The 3 groups drank comparable amounts of tea, chocolate, and cola, but smokers drank significantly more coffee than nonsmokers and ex-smokers (\( P < 0.005 \); data not shown). The self-reported weekly alcohol intake was comparable between the 3 groups.

No differences were found between the 3 groups in gestational age, induction of labor, and use of methods for pain relief, but the ways they delivered the baby were somewhat different. The number of elective cesarean sections was significantly higher and the number of vaginal deliveries was lower among ex-smokers compared with nonsmokers.

Infants born to smokers had a significantly lower birth weight and a smaller head circumference and were shorter than those born to nonsmokers and ex-smokers. Smokers also delivered a placenta of lower weight than nonsmokers and ex-smokers. The ratio of placental weight to birth weight, however, was similar for the 3 groups (data not shown).

The Apgar score at 1 and 5 minutes, infant sex ratio, and pH of umbilical cord blood were similar for newborns of nonsmokers, smokers, and ex-smokers.

**Self-Reported Smoking Status and Serum Cotinine**

In the study group of 266 women, 182 were nonsmokers and 84 were smokers before pregnancy (Table 2). The smokers were divided into 2 groups: 41 who stopped smoking at \( \approx 5 \) weeks of gestation (range, 1 to 18 weeks) and 43 who continued to smoke throughout their pregnancies. The women who continued smoking consumed a significantly higher number of cigarettes per day before pregnancy than did the women who had stopped smoking. During pregnancy, the smokers smoked significantly less than they did before pregnancy (\( P < 0.001 \)).

Detection of cotinine \( > 10 \) ng/mL is shown in Table 2. None of the samples from nonsmokers had detectable levels of cotinine, whereas 89% to 100% of the samples of smokers were cotinine positive. In samples of ex-smokers, 14% women and 5% of the fetuses were cotinine positive at \( \approx 12 \) weeks of gestation, but none of the samples was cotinine positive at \( \approx 20 \) and \( \approx 39 \) weeks of gestation.

**Lipid Profiles, Estradiol, l-Arginine, and Dimethylarginines**

Table 1 shows that at gestational week 20 maternal nonsmokers, smokers, and ex-smokers had similar plasma levels of total cholesterol, HDL, eNOS activity, and eNOS concentration logarithmically transformed because these data were highly skewed. Statistical calculations were carried out with the Stata 8.2 computer program (Stata Corp, College Station, Tex); differences were considered significant at \( P < 0.001 \).

The plasma levels of estradiol, l-arginine, ADMA, and symmetric dimethylarginine were not different among nonsmokers, smokers, and ex-smokers in either the women or the fetuses (Table 3).

**eNOS Activity and Concentration**

eNOS activity and concentration are shown in the Figure and Table 3. The same regional variation in eNOS activity and concentration was found in vessels from nonsmokers, smokers, and ex-smokers. The highest eNOS activity and concentration were found in the umbilical vein. It was therefore decided to focus on the results of that vessel because it provides the fetus with its entire blood supply.

Multiple regression analysis showed that for all the women the number of cigarettes consumed per day during pregnancy...
was negatively associated with eNOS activity ($r = -0.31$, $P < 0.001$) and eNOS concentration ($r = -0.36$, $P < 0.001$). For the pregnant smokers only, the associations with the number of cigarettes consumed per day during pregnancy with eNOS activity and concentration were $r = 0.28$ ($P < 0.001$) and $r = 0.25$ ($P < 0.001$), respectively. In addition, there was a positive association between eNOS activity and concentration ($r = 0.35$, $P < 0.001$). This latter association persisted after adjustment for smoking ($r = 0.20$, $P = 0.001$).

Maternal plasma levels of LDL were not associated with either eNOS activity or eNOS concentration in the fetal umbilical vein. The fetal plasma levels of HDL, however, were positively associated with eNOS activity ($r = 0.16$, $P = 0.012$) but not with eNOS concentration.

In regression analysis, eNOS activity in the umbilical vein exposed to maternal smoking was 36% lower (95% CI, 27 to 43), the eNOS concentration was 47% lower (95% CI, 38 to 56), and the fetal plasma level of HDL was 18% lower (95% CI, 11 to 28).

### Table 1. Clinical, Sociodemographic, and Lifestyle Characteristics of the Participants

<table>
<thead>
<tr>
<th></th>
<th>Nonsmokers (n=182)</th>
<th>Smokers (n=43)</th>
<th>Ex-Smokers (n=41)</th>
</tr>
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<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
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<tr>
<td>Age, y</td>
<td>30 (25–35)</td>
<td>30 (20–36)</td>
<td>29 (23–33)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22 (18–29)</td>
<td>24 (19–32)</td>
<td>23 (18–31)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>116 (100–135)</td>
<td>120 (81–136)</td>
<td>120 (100–136)</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>70 (60–85)</td>
<td>71 (60–85)</td>
<td>70 (60–86)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L*</td>
<td>4.9 (3.7–6.2)</td>
<td>4.9 (3.7–6.8)</td>
<td>4.7 (3.4–5.7)</td>
</tr>
<tr>
<td>LDL, mmol/L*</td>
<td>2.3 (1.2–3.4)</td>
<td>2.2 (1.3–3.1)</td>
<td>2.0 (0.9–2.9)†‡¶</td>
</tr>
<tr>
<td>HDL, mmol/L*</td>
<td>1.9 (1.3–2.4)</td>
<td>1.8 (1.3–2.5)</td>
<td>2.0 (1.5–2.7)</td>
</tr>
<tr>
<td>VLDL, mmol/L*</td>
<td>0.7 (0.4–1.3)</td>
<td>0.7 (0.4–1.3)</td>
<td>0.7 (0.4–1.2)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L*</td>
<td>1.5 (0.9–2.8)</td>
<td>1.6 (0.8–2.8)</td>
<td>1.5 (0.9–2.6)</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (1–3)</td>
<td>1 (1–4)</td>
<td>1 (1–3)</td>
</tr>
<tr>
<td>Singles, n (%)</td>
<td>0 (0)‖</td>
<td>5 (12)</td>
<td>1 (2)§</td>
</tr>
<tr>
<td>Schooling, y</td>
<td>16 (10–17)‖</td>
<td>13 (9–16)</td>
<td>16 (11–17)‡</td>
</tr>
<tr>
<td><strong>Occupational status, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>119 (65)</td>
<td>25 (58)</td>
<td>31 (76)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>11 (6)‖</td>
<td>14 (33)</td>
<td>3 (7)‖</td>
</tr>
<tr>
<td>Students</td>
<td>52 (29)‡</td>
<td>4 (9)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Caffeine intake, mg/d</td>
<td>100 (0–350)‖</td>
<td>200 (0–795)</td>
<td>150 (0–400)‡</td>
</tr>
<tr>
<td>Alcohol intake, units/wk</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>1 (0–1)</td>
</tr>
<tr>
<td><strong>Delivery, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute CS</td>
<td>9 (5)</td>
<td>2 (5)</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Elective CS</td>
<td>15 (8)</td>
<td>9 (21)</td>
<td>9 (22)¶</td>
</tr>
<tr>
<td>Vaginal</td>
<td>158 (87)</td>
<td>32 (74)</td>
<td>27 (66)¶</td>
</tr>
<tr>
<td><strong>Fetal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, d</td>
<td>281 (267–295)</td>
<td>276 (261–294)</td>
<td>280 (265–295)</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.65 (3.01–4.50)†</td>
<td>3.30 (2.54–4.14)</td>
<td>3.60 (3.06–4.55)§</td>
</tr>
<tr>
<td>Length, cm</td>
<td>52 (49–56)†</td>
<td>51 (45–54)</td>
<td>52 (49–57)§</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>36 (33–38)†</td>
<td>35 (33–37)</td>
<td>36 (33–38)‡</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>698 (516–936)§</td>
<td>628 (463–854)</td>
<td>720 (531–913)‡</td>
</tr>
<tr>
<td>1-min Apgar score</td>
<td>10 (7–10)</td>
<td>10 (7–10)</td>
<td>10 (7–10)</td>
</tr>
<tr>
<td>5-min Apgar score</td>
<td>10 (8–10)</td>
<td>10 (9–10)</td>
<td>10 (9–10)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>90 (49)</td>
<td>18 (42)</td>
<td>19 (46)</td>
</tr>
<tr>
<td>pH of cord blood</td>
<td>7.3 (7.1–7.4)</td>
<td>7.3 (7.2–7.4)</td>
<td>7.3 (7.1–7.4)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L†</td>
<td>1.3 (0.9–2.0)</td>
<td>1.2 (0.8–2.2)</td>
<td>1.1 (0.8–2.3)</td>
</tr>
<tr>
<td>HDL, mmol/L†</td>
<td>0.7 (0.4–1.2)§</td>
<td>0.6 (0.4–1.2)</td>
<td>0.7 (0.4–1.1)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L†</td>
<td>0.4 (0.2–0.8)</td>
<td>0.4 (0.2–0.8)</td>
<td>0.3 (0.2–1.0)</td>
</tr>
</tbody>
</table>

BMI indicates body mass index before pregnancy; BP, blood pressure; and CS, cesarean section. Values are medians (5th to 95th percentiles) when appropriate.

*In plasma obtained at ~20 weeks of gestation.
†In plasma from the umbilical cord.
Continuous data were analyzed by the Kruskal-Wallis test, followed by 3 pairwise comparisons of means with a Bonferroni correction to account for multiple comparisons and categorical data by χ² test: ‡P<0.05, §P<0.01, and ‖P<0.001 vs smokers; ¶P<0.05 vs nonsmokers.
CI, 9 to 26) than in nonsmokers, but similar levels were found in umbilical veins of nonsmokers and ex-smokers. (These values reflect comparisons of estimated medians.) The 36% reduction in eNOS activity became 29% after adjustment for eNOS concentration ($P<0.001$) and 27% after adjustment for fetal plasma level of HDL as well ($P<0.001$). Thus, $\approx 20\%$ of the reduction in eNOS activity between nonsmokers and smokers may be explained by differences in eNOS concentration and $\approx 5\%$ by differences in fetal plasma HDL levels.

eNOS activity was positively associated with the newborn weight ($r=0.26$, $P<0.001$); this association persisted after adjustment for body mass index before pregnancy, parity,
gestational age, and infant sex ($r=0.21, P=0.001$) and almost after adjustment for smoking as well ($r=0.12, P<0.055$). Additionally, eNOS activity was associated with the newborn weight among nonsmokers ($r=0.18, P=0.018$).

The difference in newborn weight between nonsmokers and smokers was 364 g (Table 1). After adjustment for eNOS activity, this difference was reduced to 279 g ($P=0.001$). Thus, $\approx 25\%$ of the reduction in newborn weight between nonsmokers and smokers may be explained by the differences in eNOS activity. In addition, adjusting for body mass index before pregnancy, parity, gestational age, and infant sex reduced the difference in newborn weight between nonsmokers and smokers to 242 g ($P=0.002$).

The ratio of eNOS activity to eNOS concentration (the specific eNOS activity) was similar for nonsmokers, smokers, and ex-smokers without a significant relationship to smoking history among smokers (data not shown). The significantly lower values of eNOS activity and concentration in the umbilical vein from smokers compared with nonsmokers and ex-smokers were present in both male and female infants (data not shown).

**Discussion**

The present study confirms previous findings that newborns of smokers have reduced fetal growth as measured by weight, head circumference, and length compared with newborns of nonsmokers and ex-smokers without a significant relationship to smoking history among smokers (data not shown). The significantly lower values of eNOS activity and concentration in the umbilical vein from smokers compared with nonsmokers and ex-smokers were present in both male and female infants (data not shown).

In the present study, the smaller fetal outcome and lower eNOS activity and concentration persisted after adjustment for maternal sociodemographic and lifestyle characteristics, including caffeine intake.23–25

In an observational study like this, confounding is possible because the smokers differ from each of the 2 other groups by a number of measured characteristics26,27 and probably also by some unmeasured or immeasurable characteristics. In the absence of randomized studies to determine the cause/effect of smoking on the fetus, the observation that ex-smokers had the same eNOS activity and fetal characteristics as nonsmokers adds evidence to the hypothesis that smoking is a direct cause of the observed changes in the newborns of smokers. There are thus 3 different possibilities: (1) Smoking reduces fetal eNOS activity and reduces birth weight by 2 separate and different mechanisms; (2) smoking reduces birth weight by a mechanism that consequently also causes lower eNOS activity (eg, smaller cells all over the fetus, including ECs); or (3) smoking reduces eNOS activity that consequently leads to a lower birth weight.

The similar specific eNOS activity in the 3 groups suggests that smoking reduces eNOS activity at least in part by reducing eNOS concentration. Multiple regression analysis suggested that 20% of the reduction in eNOS activity associated with smoking may be explained by a reduced eNOS concentration and 5% by a reduced fetal plasma level of HDL. Reduced plasma level of HDL has been associated with impaired endothelium-dependent NO-mediated vasodilation in human brachial arteries.28 Furthermore, HDL increased eNOS activity in human EC cultures.29,30 Despite these findings, a causal relationship between HDL levels and eNOS activity in humans has not yet been demonstrated. The remaining 75% reduction in eNOS activity may thus be explained by a separate effect of smoking directly on eNOS activity.

Several studies demonstrate a lower bioavailability of estrogens, including estradiol, in nonpregnant smoking women,31,32 and animals treated with estradiol show increased eNOS activity.20 The effect of smoking on the eNOS inhibitor ADMA remains controversial.33,34 Apparently, the present reduction in eNOS activity associated with smoking in pregnancy was not mediated by changes in plasma levels of estradiol,35 ADMA, and the eNOS substrate L-arginine.36
Nicotine is among the numerous compounds contained in cigarette smoke that could influence the altered vascular reactivity in smokers. In cigarette replacement studies, a higher fetal umbilical artery blood flow velocity and a somewhat higher fetal aortic blood flow velocity were found in pregnant smokers chewing nicotine gum compared with their baseline values. Furthermore, acute inhibition (within 10 minutes) of endothelium-derived NO bioactivity in human hand veins is found after infusion of nicotine corresponding to plasma concentrations obtained by smoking a cigarette. Considered together, these findings suggest that nicotine acts directly on the endothelium, causing vasoconstriction by inhibition of eNOS activity.

It is well known that a higher carbon monoxide (CO) level in the blood, which reduces the oxygen-carrying capacity of the blood, follows cigarette smoking. Because of the low fetal oxygen saturation, the presence of carboxyhemoglobin level has a more serious effect on fetal blood oxygen transport than it does on maternal blood oxygen transport. This has previously led investigators to suggest that ultrastructural abnormalities of ECs in the umbilical vein from smoking mothers were due primarily to hypoxia caused by CO exposure. When human umbilical vein ECs were cultured under hypoxic conditions (an oxygen level <5%), eNOS activity and concentration were significantly lower compared with those cultured under a normal oxygen level (21%).

In addition, it has been suggested that cigarette smoking induces oxidative stress, eg, by enhancing the oxidation of LDL, and that oxidized LDL impairs forearm blood flow and endothelium-dependent vasorelaxation in rabbit aorta. However, to what extent maternal smoking reduces eNOS activity by nicotine, CO, or oxidized LDL in the fetal circulation warrants further investigations.

Among the 182 nonsmokers in the present study, we observed a significant positive association between eNOS activity and birth weight, suggesting that eNOS activity is important for the weight of the newborn. However, only 25% of the reduction in newborn weight was explained by the reduced eNOS activity. That means that 75% of the reduction in birth weight is explained by other mechanisms of smoking besides reduced eNOS activity, eg, a direct effect of nicotine, CO, and other toxic substances in cigarette smoke on pathways not including the production of NO.

An important limitation of the present observational study is the difficulty in elucidating causality between various variables, as mentioned above. Another important limitation is that we do not know to what extent the eNOS activity measured with the citrulline assay reflects the in vivo production of NO. It is thus possible that the reduction in eNOS activity measured in the present study with optimized addition of various cofactors reflects only a fraction of the reduction of NO production that occurs in vivo in the fetal vessels during smoking by the pregnant woman. Thus, potential effects of smoking related to alterations in tissue cofactor availability may be missed, and only a reduction in eNOS activity as a result of lower eNOS concentration related to smoking may be detected by this method.

Conclusions

The reduction in size and in eNOS activity and concentration in umbilical and chorionic endothelium of the newborns of women who smoke may be prevented by smoking cessation early in pregnancy. The findings suggest that smoking in pregnancy reduces the endothelial production of NO in the fetal vessels, contributing to restricted fetal growth caused by an impaired vasodilatory capacity, and that smoking cessation early in pregnancy prevents these effects on the newborn.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

Newborns of smokers have reduced weight, length, and head circumference compared with newborns of nonsmokers and newborns of women who stopped smoking early in pregnancy. The mechanisms behind this effect have not yet been elucidated. Endothelial cells produce the vasodilator nitric oxide (NO) synthesized by the enzyme endothelial NO synthase. NO is of fundamental importance for regulating the tone of vascular smooth muscle cells and thereby the blood flow through the vessels. Reduced production of NO in fetal vessels in pregnant smokers may lower the blood flow to the fetus and result in retarded fetal growth. The present study measured endothelial NO synthase activity in freshly isolated endothelial cells from umbilical and chorionic vessels of 182 nonsmokers, 43 smokers, and 41 women who stopped smoking early in pregnancy and related the findings to the fetal outcome. eNOS activity in fetal umbilical and chorionic vessels exposed to maternal smoking was significantly lower than in unexposed fetal vessels, and enzyme levels were similar in the fetal vessels of women who stop smoking early in pregnancy compared with those of nonsmoking mothers. It is unknown whether maternal smoking is a risk factor for subsequent endothelial dysfunction and ischemic heart disease in the offspring. Nicotine and carbon monoxide are among the numerous compounds contained in cigarette smoke that may alter the reactivity in the fetal vessels. The harmful substances, however, have not yet been identified. Our results add evidence to the importance of smoking cessation early in pregnancy.
Smoking Cessation Early in Pregnancy and Birth Weight, Length, Head Circumference, and Endothelial Nitric Oxide Synthase Activity in Umbilical and Chorionic Vessels: An Observational Study of Healthy Singleton Pregnancies
Malene R. Andersen, Ulf Simonsen, Niels Uldbjerg, Christian Aalkjær and Steen Stender

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