Maternal smoking is associated with mitochondrial DNA depletion and respiratory chain complex III deficiency in placenta

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PREVALENCE STUDIES SUGGEST that 20–50% of women from developed countries report smoking at the onset of pregnancy (15). It is well known that maternal smoking during pregnancy is associated with adverse outcomes, among which intrauterine growth retardation (IUGR) is the most frequent complication (1, 15, 20, 41, 48). Infants born to smoking mothers are on average 200 g lighter at birth than the infants of nonsmokers, even after adjustment for gestational age (1, 41). This effect on fetal growth occurs during the third trimester of pregnancy in a dose-dependent manner (41), although the specific mechanisms by which maternal smoking affects fetal growth have not yet been fully understood (1, 41).

The first proposed mechanism is a uteroplacental insufficiency, which results either from a nicotine-mediated reduction in uteroplacental blood flow or from structural changes in the placenta (e.g., placental infarcts) (1, 41). Second, maternal smoking may induce fetal hypoxia through diffusion of carbon monoxide (CO), nicotine, and thiocyanate across the placenta (1, 41). Third, changes in the transplacental transport of amino acids or zinc have been proposed as possible explanations for the impact of cigarette smoking on fetal growth (1, 38). Finally, various chemical components within tobacco smoke exert direct effects on fetal and placental cells (41). These toxic agents, including carcinogen and free radical-forming substances, may cause extensive damage to DNA, proteins, and lipids, as well as to organelles like mitochondria.

The adverse effects of smoking on mitochondrial respiratory function have already been described in tissues such as cardiomyocytes (decrease in stimulated respiration) (14), lymphocytes (decrease in complex IV activity) (32), and monocytes (disruption of mitochondrial membrane potential and apoptosis) (3). To date, however, no study has investigated the effects of smoking on placental mitochondria.

Our hypothesis was that maternal smoking could alter placental mitochondrial function. We evaluated the respiratory chain capacity of the human term placental mitochondria of smoking mothers by measuring both oxygen consumption and respiratory complex activities. Moreover, using a real-time quantitative PCR method for the quantification of mitochondrial DNA (mtDNA) with respect to the β-globin gene, we estimated the relative content in mtDNA in placenta.

MATERIALS AND METHODS

Subjects

This study was approved by the ethics committee of the University of Angers. Placentas were obtained with informed consent of the parents.

Mothers. Over a 6-mo period, 28 healthy mothers who had given birth to healthy singletons by elective cesarean delivery planned before the onset of labor were included in the study. Nine were smokers and 19 were nonsmokers. The smoking mothers reported a cigarette consumption of 10.2 (range 3–20) cigarettes per day. None of the mothers had preeclampsia, diabetes, or hypertension, and all denied the use of any illegal substance. Subjects with complications during pregnancy were excluded.

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Newborns. Nine full-term infants delivered by mothers who had smoked during pregnancy and 19 full-term infants whose mothers were nonsmokers were studied. All the neonates were healthy, had no signs of fetal distress, and had Apgar scores of 7 or more at 1 and 5 min. The Apgar score assesses the condition of newborn infants and focuses on five objective clinical signs: heart rate, respiratory effort, muscle tone, reflex irritability, and color (35). Newborn gestational age (37–42 wk) was determined with the use of ultrasonographic criteria from early ultrasound scan. Body weight and length were recorded. The percentile for birth weight was calculated according to the standard French growth curves of Leroy and Lefort (23).

Isolation of Mitochondria from Human Placenta

Placentas were collected within 15 min of delivery. Human placental mitochondria were obtained with the modified protocol of differential centrifugation previously described by Martinez et al. (27). Briefly, several placental cotyledons were removed from the maternal side of the placenta and placed immediately into an ice-cold medium containing 250 mM sucrose and 1 mM EDTA, pH 7.4. All steps were carried out at 4°C. Soft villous tissue (24–36 g) was freed of maternal side of the placenta and placed immediately into an ice-cold medium and homogenized with a Potter-Elvehjem homogenizer (7 up-and-down strokes). After further filtration on a surgical gauze membrane was found to be 5.7% for both groups.


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\textsuperscript{9}\textsuperscript{H}11006
\textsuperscript{10}\textsuperscript{H}11006
\textsuperscript{11}\textsuperscript{H}11006
\textsuperscript{12}\textsuperscript{H}11006

Table 1. Comparison of respiratory parameters for isolated placental mitochondria stimulated by ADP with or without succinate in the conservation buffer

<table>
<thead>
<tr>
<th>O\textsubscript{2} Consumption, nmol O\textsubscript{2}-min\textsuperscript{-1}mg mitochondrial protein\textsuperscript{-1}</th>
<th>With succinate (n = 9)</th>
<th>Without succinate (n = 9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>State 2</td>
<td>11.08 ± 2.12</td>
<td>10.01 ± 4.55</td>
<td>0.547</td>
</tr>
<tr>
<td>Inhibition by rotenone</td>
<td>10.48 ± 1.9</td>
<td>9.6 ± 1.63</td>
<td>0.326</td>
</tr>
<tr>
<td>State 3</td>
<td>37.18 ± 14.8</td>
<td>36.39 ± 27.2</td>
<td>0.943</td>
</tr>
<tr>
<td>State 4-oligomycin</td>
<td>9.43 ± 2.1</td>
<td>9.75 ± 4.62</td>
<td>0.865</td>
</tr>
<tr>
<td>Uncoupled FCCP</td>
<td>84.26 ± 21.26</td>
<td>61.43 ± 33.77</td>
<td>0.130</td>
</tr>
<tr>
<td>RCI</td>
<td>3.94*</td>
<td>3.73*</td>
<td>0.711</td>
</tr>
</tbody>
</table>

O\textsubscript{2} consumption values are means ± SE. Placental mitochondria were isolated by differential centrifugation using the method previously described by Martinez et al. (27) and then resuspended in a minimal volume of distilled water before the addition of 10 mM KH\textsubscript{2}PO\textsubscript{4}, pH 7.5, 2 mM KCN, 2 μg/ml antimycin A, 100 μM decylubiquinone, 1 mg/ml BSA, and 5.2 mM MgCl\textsubscript{2}. The reaction was then initiated by the addition of 200 μM NADH, and the decrease in the optical density due to the oxidation of NADH was measured at 340 nm for 3 min. The NADH ubiquinone c reductase activity was also measured in the presence of 2 μg/ml rotenone. The enzymatic activity of complex I was deduced.
from the difference between NADH oxidation activity with and without rotenone.

Activity of complex II (succinate dehydrogenase). The activity of succinate dehydrogenase was measured at 600 nm by following the reduction of 2,6-dichlorophenol-indophenol (DCPIP) in the presence of phenazine methosulfate (PMS). Isolated mitochondria (5 μl) were preincubated in a buffer containing 50 mM KH₂PO₄, pH 7.5, 16 mM of oxidized cytochrome c, 1.5 mM KCN, and 100 μM PMS for 5 min at 37°C. The reaction was then started by the addition of 100 μM DCPIP, and the optical density was analyzed for 3 min.

Activity of complex III (ubiquinol cytochrome c reductase). Complex III activity was determined by measuring the reduction of oxidized cytochrome c at 550 nm. Isolated mitochondria (5 μl) were incubated for 30 s at 37°C in a reaction medium containing 35 mM KH₂PO₄, pH 7.5, 100 mM KCN, 1.8 mM KCN, 125 mM oxidized cytochrome c, and 10 μg/ml rotenone. The reaction was initiated by the addition of 31.8 μM decylubiquinol, which had been prepared as previously described by Malgat et al. (26). The increase in the optical density was recorded for 3 min. The reduction of cytochrome c was also measured after addition of 10 μg/ml antimycin A. The enzymatic activity of complex III was calculated from the difference between the cytochrome c reduction reaction with and without antimycin A.

Activity of complex IV (cytochrome c oxidase). The cytochrome c oxidase activity was estimated by recording the reduction of oxidized cytochrome c at 550 nm. A 50 μM solution of reduced cytochrome c (92–97% reduced using dithionite) in 10 mM KH₂PO₄, pH 7, was preincubated for 5 min at 37°C. The reaction was initiated by addition of the isolated mitochondria (5 μl), and the change in optical density was measured for 1.5 min.

Activity of complex II+III (succinate cytochrome c reductase). The activity of complex II+III was measured by observing the reduction of oxidized cytochrome c at 550 nm. The reaction medium, which contained 9 mM KH₂PO₄, pH 7.5, 100 μM oxidized cytochrome c, 1 mM KCN, 200 μM ATP, 4 μg/ml rotenone and isolated mitochondria (5 μl), was preincubated at 37°C for 30 s. The reaction was initiated by adding 5 mM succinate, and the change in optical density was measured for 2.5 min.

As for oxygen consumption, we have previously checked that there was no difference in the respiratory complex activities whether we used succinate in the conservation buffer or not (Table 2).

Table 2. Respiratory complex activities of isolated placental mitochondria with or without succinate in the conservation buffer

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>With succinate (n = 9)</th>
<th>Without succinate (n = 9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>145.04±47.74</td>
<td>108.39±54.76</td>
<td>0.193</td>
</tr>
<tr>
<td>Complex II</td>
<td>83.51±34.46</td>
<td>72.3±15.67</td>
<td>0.409</td>
</tr>
<tr>
<td>Complex III</td>
<td>133.63±44.17</td>
<td>110.87±45.67</td>
<td>0.299</td>
</tr>
<tr>
<td>Complex II+III</td>
<td>314.42±125.21</td>
<td>276.26±62.62</td>
<td>0.425</td>
</tr>
<tr>
<td>Complex IV</td>
<td>445.74±199.39</td>
<td>579.38±123.51</td>
<td>0.114</td>
</tr>
<tr>
<td>CS</td>
<td>667.56±166.39</td>
<td>714.82±203.31</td>
<td>0.597</td>
</tr>
</tbody>
</table>

Enzymatic activity values are means ± SE. Placental mitochondria were isolated by differential centrifugation using the method described by Martinez et al. (27), resuspended in a minimal volume of respiratory medium containing, or not, 10 mM succinate, and then stored at −80°C until analysis. Activities of citrate synthase (CS) and complexes I, II, III, and IV+III were measured spectrophotometrically at 37°C with modified procedures of Malgat et al. (26), in agreement with the Mitochondrial Diseases Group of the Association Française contre les Myopathies. We used unpaired Student’s t-tests for comparisons between groups.

mtDNA/β-Globin Real-Time PCR Quantification

DNA was extracted from each placental sample of smoking mothers and nonsmoking controls by the conventional phenol-chloroform method. Quantification of mtDNA/cell was performed using a mtDNA/β-globin real-time PCR quantification method as previously described (29). Briefly, a Roche LightCycler was used to determine the mtDNA copy number using the LightCycler-Faststart DNA Master SYBR Green 1 Kit (Roche, Mannheim, Germany). PCR reaction mixtures with a total volume of 20 μl were prepared as follows: 1× buffer containing 4 mM MgCl₂, 0.2 mM dNTP, 0.5 μM of both primers (D14 and R56), SYBR green I dye, 0.25 IU of hot start Taq DNA polymerase, and 2 μl of the extracted DNA or 2 μl of standard with a known copy number. The nucleotide positions of the primers on the light-strand mtDNA (according to the Cambridge reference sequence) were D41 (3254–3277) and D56 (3126–3147). The external standard used for mtDNA quantification was the corresponding 158-bp PCR product cloned into PCR 2.1-TOPO vector (Invitrogen, Life Technologies, Groningen, The Netherlands). The reactions were performed as follows: initial denaturing at 95°C for 7 min and 40 cycles at 95°C for 1 s, 55°C for 5 s, and 72°C for 13 s. The SYBR green fluorescence was read at the end of each extension step (72°C). A melting curve (loss of fluorescence at a given temperature between 66 and 94°C) was analyzed to check the specificity of the PCR product. For each run, a standard curve (log of the initial template copy no. on the abscissa and the cycle no. at the crossing point on the ordinates) was generated using five 10-fold serial dilutions (10–100,000 copies) of the external standard. This curve enabled the determination of the starting copy number of mtDNA in each sample. To determine the number of cells in each sample, we quantified the β-globin gene by means of the LightCycler-Control Kit DNA (Roche) according to the manufacturer’s recommendations. A 110-bp fragment of the β-globin gene was amplified using human genomic DNA as a standard. Eighteen microliters of PCR reaction mixture were added to 2 μl of the extracted DNA or to 2 μl of standard with a known β-globin copy number. The reactions were performed on the LightCycler as follows: initial denaturing at 95°C for 7 min and 40 cycles at 95°C for 1 s, 55°C for 5 s, and 72°C for 10 s. The SYBR green fluorescence was read at the end of each extension step (72°C). The melting curve analysis was systematically performed. For each run, a standard curve was generated using five 10-fold serial dilutions (1–10,000 copies) of the standard. The standard curve generated by the LightCycler software (version 3.5) enabled the determination of the starting copy number of β-globin gene in each sample. All samples were tested in duplicate. The calculation of the mtDNA/cell ratio was performed taking into account the diploidy of the placental cells (mtDNA/2β-globin). We previously demonstrated that the PCR efficiency of this method is constant over the concentration range studied (10–100,000 copies) and is almost the same for the standards as for the DNA extracts (29). The intra- and interassay coefficients of variation for the mtDNA and β-globin quantification were between 5.5 and 8.9% and between 6.5 and 12.8%, respectively.

Blood Sampling

Venous cord blood was drawn immediately after birth for measurements of IGF-I and insulin-like growth factor-binding proteins-3 and -1 (IGFBP-3 and IGFBP-1, respectively). Serum IGF-I measurements were performed by immunoradiometric assay (IRMA) after acid-ethanol extraction; IGFBP-3 and IGFBP-1 measurements were made by IRMA (Immunotech/Beckman Coulter, Villepinte, France, for IGFBP-3, and DSL, Webster, TX, for IGFBP-1). The intra- and interassay coefficients of variation were 5.7 and 8.6% for IGF-I and 4.8 and 6.4% for IGFBP-3, respectively.
**Statistical Analysis**

Results were expressed as means ± SE. We used unpaired Student’s t-tests for comparisons between groups. Simple correlation analyses were performed using Pearson’s correlation coefficient. Significance was defined as \( P < 0.05 \). All analyses were two tailed and performed with the StatView 5.0 statistical package.

**RESULTS**

**Auxological Characteristics**

The auxological characteristics of mothers, newborns, and placentas are summarized in Table 3. The smoking and nonsmoking mothers had similar maternal characteristics. As expected, birth weight, birth length, and placental weight tended to be lower in the neonates from mothers who had smoked during pregnancy; however, these differences did not reach statistical significance.

**Effects of Maternal Smoking on Oxygen Consumption of Isolated Mitochondria**

Table 4 shows the oxygen consumption of isolated human term placental mitochondria from smoking (\( n = 9 \)) and nonsmoking (\( n = 19 \)) mothers. Smoking during pregnancy did not change the sensitivity of isolated mitochondrial to ADP [state 4, 10.32 ± 6.1 vs. 9.55 ± 3.12 nmol O2·min⁻¹·mg⁻¹; state 3, 38.33 ± 17.33 vs. 36.87 ± 19.74 nmol O2·min⁻¹·mg⁻¹; and RCI, 3.71 ± 3.86, for placentas of smoking and nonsmoking mothers, respectively; not significant (NS)]. The maximal oxygen uptake observed after addition of FCCP, a potent uncoupler, to the respiratory medium was similar in both categories (93.27 ± 37.73 vs. 75.13 ± 28.28 nmol O2·min⁻¹·mg⁻¹ for smoking- and nonsmoking-exposed placentas, respectively; NS).

**Effects of Maternal Smoking on the Respiratory Complex Activities of Human Placental Mitochondria**

Table 5 shows the results of the mitochondrial respiratory chain enzyme activities in the placentas from smoking and nonsmoking mothers. The enzymatic activities are either expressed in nanomoles per minute and per milligram of mitochondrial protein or referenced to CS activity. In both cases, the enzymatic activity of complex III was significantly reduced in placental mitochondria from smoking mothers (87.32 ± 19.89 vs. 122.51 ± 43.92 nmol·min⁻¹·mg⁻¹, \( P = 0.03 \), or 0.129 ± 0.051 vs. 0.183 ± 0.063, \( P = 0.03 \), for smoking- and nonsmoking-exposed placentas, respectively). Furthermore, a significant negative relationship was observed between the number of cigarettes smoked per day and the degree of impaired complex III enzymatic activity (\( r = −0.34 \) and \( P < 0.05 \)).

In contrast, other respiratory complex activities of the placental mitochondria were not significantly different between the smoking and nonsmoking categories.

**Effects of Maternal Smoking on the mtDNA Content**

The average mtDNA/β-globin ratio was reduced by 37% in the smoking group vs. the control group (2.119 ± 294 vs. 3.323 ± 1.954 mtDNA copy/cell, respectively; \( P < 0.02 \)). Furthermore, a significant negative relationship was found between the number of cigarettes smoked per day and the relative content in mtDNA (\( r = −0.42 \), \( P < 0.05 \)).

**Correlations Between Cord Blood IGF-I, IGFBP-3, and IGFBP-1 and Placental Mitochondrial Function**

Although mean birth weight was 200 g lighter in newborns from smoking mothers compared with nonsmoking mothers, this did not reach significance. Similarly, a trend toward negative correlation was observed between birth weight and complex III activity or mtDNA content. Because the lack of significance may have been due to the small size of the groups, we also studied the relationships between the mitochondrial alterations and three biological markers reflecting fetal nutrition and growth, namely cord blood IGF-I, IGFBP-3, and IGFBP-1.

Cord blood IGF-I was positively correlated with birth weight (\( r = 0.69 \), \( P < 0.01 \)) and cord blood IGFBP-3 (\( r = 0.48 \), \( P = 0.05 \)) and negatively correlated with cord blood IGFBP-1 (\( r = −0.63 \), \( P < 0.01 \)). Cord blood IGF-I was significantly lower in the newborns from smoking than from nonsmoking mothers: 98 ± 19 vs. 125 ± 20 μg/l (\( P < 0.05 \)). Cord blood IGFBP-3 showed a nonsignificant trend toward lower values in the newborns from smoking vs. nonsmoking mothers: 1,160 ± 90

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**Table 3. Auxological characteristics of mothers, placentas, and newborns according to maternal smoking category**

<table>
<thead>
<tr>
<th></th>
<th>Nonsmoking (( n = 19 ))</th>
<th>Smoking (( n = 9 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age, yr</td>
<td>30.6 ± 4.9</td>
<td>30.6 ± 6.4</td>
</tr>
<tr>
<td>Wt gain, kg</td>
<td>13.1 ± 5.3</td>
<td>13.3 ± 5.6</td>
</tr>
<tr>
<td>Gestation no.</td>
<td>2.8 ± 1.3</td>
<td>3.4 ± 2.7</td>
</tr>
<tr>
<td><strong>Newborns</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>38.7 ± 1.1</td>
<td>38.7 ± 1.1</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>10/9</td>
<td>4/5</td>
</tr>
<tr>
<td>Birth wt, g</td>
<td>3.26 ± 0.268</td>
<td>2.99 ± 0.560</td>
</tr>
<tr>
<td>Birth wt, percentile</td>
<td>51 ± 25</td>
<td>38 ± 30</td>
</tr>
<tr>
<td><strong>Placentas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental wt, g</td>
<td>607 ± 68</td>
<td>571 ± 85</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. M, male; F, female.
vs. 1.240 ± 110 μg/l (P = 0.10). Cord blood IGFBP-1 was not statistically different in the newborns from smoking compared with nonsmoking mothers; 47.9 ± 16 vs. 46.4 ± 30 μg/l (NS). We also observed a negative correlation between the number of cigarettes smoked per day and cord blood IGF-I (r = −0.571 and P < 0.05).

mtDNA content showed a trend toward positive correlation with cord blood IGF-I (r = 0.20, P = 0.12) and positive correlation with cord blood IGFBP-3 (r = 0.74, P < 0.01) but no correlation with cord blood IGFBP-1. Complex III enzymatic activity was not related to cord blood IGF-I, IGFBP-3, or IGFBP-1.

**DISCUSSION**

We examined the effects of tobacco smoke exposure during pregnancy on the respiratory chain of human term placental mitochondria. We observed a significant reduction (up to 29%) of the enzymatic activity of complex III in placental mitochondria from smoking mothers compared with nonsmoking mothers, with a negative correlation between complex III activity and the number of cigarettes smoked per day. We also noted a significant decrease in the relative content of mtDNA in the placenta of smokers, which was also negatively correlated with the number of cigarettes smoked per day. These modifications could alter the placental energy-producing system and contribute to the reduced birth weight in newborns of smoking mothers.

The inhibition of enzymatic complex activity has been observed in other tissues from smokers, such as skeletal muscle and lymphocytes (32, 33). In this study, we found a significant decrease in the complex III activity in placenta from smoking mothers. Moreover, this activity was inversely correlated with the number of cigarettes smoked per day. However, complex II+III activity was unchanged. This apparent discrepancy may be related to the low sensitivity of this measurement, since it has been reported that measurements of complex II+III activity in the muscle failed to detect partial deficiency of complex III (43). The significant reduction in complex III activity did not impair the oxygen consumption of isolated placental mitochondria in this study. However, it has been shown that the activity of a respiratory chain complex can be inhibited, up to a critical value, without affecting the rate of mitochondrial respiration or ATP synthesis, a phenomenon called the mitochondrial threshold effect (24, 26, 30, 40). Alternatively, normal in vitro measurements do not preclude the possibility of decreased in vivo activities, since oxygen consumption measurements, which involve several complexes, constitute a factor with low discriminative power in the analysis of respiratory complex deficiencies (26). Finally, whether the decrease in complex III activity we found has a significant impact on mitochondrial function remains to be determined.

How maternal smoking could specifically decrease the complex III activity in placenta is unknown, although several hypotheses can be advanced on the basis of the known tobacco-related alterations of mitochondrial function in other tissues. Several components of tobacco smoke could be responsible for such an inhibition. For instance, cadmium, which is present in high concentrations in cigarette smoke and can accumulate in placenta (17, 18, 37), was found to inhibit mitochondrial electron transport at the level of complex III in rat liver (31). Defects that cadmium could cause between NAD-linked substrates and the respiratory chain could constitute a protecting mechanism of mitochondria by limiting electron flow into complex III (6). Cadmium, along with other harmful components of cigarette smoke, has also been shown to disrupt placental hormone synthesis (1, 18, 19, 25, 37, 41). Alternatively, the decreased complex III activity we observed could have been due to the adverse effects of reactive oxygen species (ROS). ROS from exogenous and endogenous sources are increased in smokers (32). ROS production has been shown to cause a decrease in complex III activity by inducing cardiopulmonary peroxidation in mitochondria isolated from rat heart subjected to ischemia and reperfusion (36). Future studies are needed to further explore these hypotheses.

We also observed that the relative content in mtDNA was decreased in the placentas of smokers. Because comparable CS activity was found in the homogenates of the two groups, this result may be interpreted as a decrease in the number of genomes per mitochondrion rather than a decrease in the number of mitochondria. Moreover, the relative content in mtDNA was negatively correlated with the number of cigarettes smoked every day; thus, suggesting that maternal smoking during pregnancy modulates mtDNA replication in a negative manner. This finding agrees with a recent study that demonstrated a decrease in the mtDNA content in the lung of smokers, which was attributed to the oxidative stress induced by smoking (22). Exposure to cigarette smoke induces DNA damage, as indicated by the ability of a large number of chemical components of tobacco smoke to form covalent addition products (adducts) with DNA (7, 9, 38). In the lung, the DNA adduct 8-OHdG was found to be increased whereas mtDNA was decreased (22). In the placentas of smoking mothers, which was attributed to the oxidative stress induced by smoking (22), ROS from exogenous and endogenous sources are increased in smokers (32). ROS production has been shown to cause a decrease in complex III activity by inducing cardiopulmonary peroxidation in mitochondria isolated from rat heart subjected to ischemia and reperfusion (36). Future studies are needed to further explore these hypotheses.
maternal smoking. The implication of such alterations in the mitochondrial function and mtDNA content in the placentas from smoking mothers could thereby affect active nutrient transfer and contribute to the impairment of fetal growth. The changes we observed in mitochondrial function and mtDNA content in the placentas from smoking mothers compared with nonsmoking mothers suggest, but do not prove, a causal link between smoking and these modifications. Notably, the number of cigarettes smoked per day was negatively correlated with the enzymatic activity of complex III as well as with placental mtDNA content, which suggests a dose effect. Although the mean difference in birth weight between the infants from the smoking and nonsmoking mothers was 200 g, in agreement with epidemiological studies (1, 41), this was not significant, likely because of the small size of our cohort. To further study mitochondrial alterations in relation to fetal nutrition and growth, we measured cord blood IGF-I, IGFBP-3, and IGFBP-1. IGF-I and IGFBP-3, which mainly arise from the fetal liver, are known to be decreased, and IGFBP-1 increased, in case of reduced availability of substrates or oxygen to the fetus, irrespectively of the cause of the nutrient deficit (2, 11, 13, 39, 47). The decrease in cord blood IGF-I and the trend for a decrease in cord blood IGFBP-3 likely indicated a reduced placental supply of nutrient in smoking-exposed newborns, in agreement with previous studies (11, 13, 47). Furthermore, the positive correlation between mtDNA content and cord blood IGFBP-3 and the trend toward positive correlation with cord blood IGF-I supported a link between placental mitochondrial alterations and nutrient supply to the fetus. Although cord blood IGFBP-1 and IGF-I were negatively correlated, no relationship was found between cord blood IGFBP-1 and birth weight or cigarette smoking in our study, possibly because IGFBP-1 is less directly linked to fetal growth than IGF-I (47). Overall, this suggests, but does not prove, a causal association between placental mitochondrial alterations, nutrient supply to the fetus, and the production of hormones and proteins directly or indirectly involved in fetal growth.

In conclusion, we showed significant alterations in mitochondrial function and mtDNA content in the placentas from smoking mothers. The implication of such alterations in the restricted growth of fetuses from smoking mothers remains to be determined. The reduction in complex III enzymatic activity and the decrease in the relative content in mtDNA could limit energy availability in trophoblastic cells. As a result, nutrient transfer to the fetus and protein synthesis required for the development of the placenta might be impaired, resulting in restricted growth. If this is proven to be true, it might have implications for management of pregnant smokers: benefits could be expected from dietary supplements known to support energy productions, ranging from antioxidant therapy (5) to nicotine replacement therapy in women who continue to smoke despite medical advice to stop. Currently, clinical trials are in progress to evaluate some of these treatment options and their effect on fetal growth.

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REFERENCES


