Cigarette exposure induces changes in maternal vascular function in a pregnant mouse model

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Gandley RE, Jeyabalan A, Desai K, McGonigal S, Rohland J, DeLoia JA. Cigarette exposure induces changes in maternal vascular function in a pregnant mouse model. Am J Physiol Regul Integr Comp Physiol 298: R1249–R1256, 2010. First published February 17, 2010; doi:10.1152/ajpregu.00274.2009.—Smoking is associated with multiple adverse pregnancy outcomes, including fetal growth restriction. The objective of this study was to determine whether cigarette smoke exposure during pregnancy in a mouse model affects the functional properties of maternal uterine, mesenteric, and renal arteries as a possible mechanism for growth restriction. C57Bl/CJ mice were exposed to whole body sidestream smoke for 4 h/day. Smoke particle exposure was increased from day 4 of gestation until late pregnancy (day 16–19), with mean total suspended particle levels of 63 mg/m³, representative of moderate-to-heavy smoking in humans. Uterine, mesenteric, and renal arteries from late-pregnant and virgin mice were isolated and studied in a pressure-arteriograph system (n = 23). Plasma cotinine was measured by ELISA. Fetal weights were significantly reduced in smoke-exposed compared with control fetuses (0.88 ± 0.1 vs. 1.0 ± 0.08 g, P < 0.02), while litter sizes were not different. Endothelium-mediated relaxation responses to methacholine were significantly impaired in both the uterine and mesenteric vascularature of pregnant mice exposed to cigarette smoke during gestation. This difference was not apparent in isolated renal arteries from pregnant mice exposed to cigarette smoke; however, relaxation was significantly reduced in renal arteries from smoke-exposed virgin mice. In conclusion, we found that passive cigarette smoke exposure is associated with impaired vascular relaxation of uterine and mesenteric arteries in pregnant mice. Functional maternal vascular perturbations during pregnancy, specifically impaired peripheral and uterine vasodilation, may contribute to a mechanism by which smoking results in fetal growth restriction.

intrauterine growth restriction; passive smoke; vasorelaxation; arterial relaxation; myogenic reactivity

IN 2005, over 20 million (18.1%) American women smoked cigarettes (5). While ~25% of smoking women quit after becoming pregnant, an estimated 10.7% of all pregnant women continue to smoke during pregnancy. The impact of smoking on reproductive-aged women includes reductions in fertility and increases in early pregnancy loss, as well as complications later in pregnancy, including spontaneous miscarriage, low birth weight, prematurity, intrauterine growth restriction, placental abruption, perinatal death, and postnatal morbidity (1, 44, 68). Premature delivery and low birth weight are common among smoking mothers and remain a primary cause of neonatal death (4). Approximately 65% of infant deaths occur among infants with birth weight <2,500 g (26, 27). Maternal smoking remains the single largest modifiable risk factor for intrauterine growth restriction (4, 12, 33, 34, 39–41, 53, 61, 69).

Pregnancy is characterized by profound changes in the maternal vasculature, particularly the uterine circulation (49). Vascular adaptation to pregnancy includes both dilatation and angiogenesis (47). Systemic cardiovascular changes occur early in normal pregnancy, with a marked decline in peripheral vascular resistance and blood pressure, an increase in cardiac output and blood volume, and a decrease in perfusion pressure (14). The marked reduction in systemic vascular resistance occurs at the level of the resistance vessels, with an increase in cross-sectional area and vessel compliance. Vascular responsiveness to pressors such as angiotensin II and noradrenaline is attenuated, and vasodilatory responses are enhanced via an endothelium-dependent mechanism (22, 32, 45). Changes in the renal circulation are initiated even prior to pregnancy in the luteal phase of the menstrual cycle. By 4–6 wk of gestation, renal blood flow and glomerular filtration rate are increased to 20% above baseline (15). Overall, effective renal plasma flow increases by 50–85% during gestation (14). Unlike changes in uterine blood flow that occur relatively late in pregnancy, reduced total peripheral vascular resistance, increased cardiac output, and increased renal blood flow are early changes that anticipate the needs of the growing uterus, placenta, and fetus. The mechanisms underlying the functional and structural changes of the uterine and peripheral arteries during early- and late-pregnancy vascular adaptations are not fully recognized. Striking similarities in the uteroplacental vascular structure, as well as the response of peripheral vascular beds to pregnancy, have been found between humans and other species with hemochorial-type placenta (including rodents) (2, 13, 14, 47, 50). Associations between fetal growth restriction and inadequate plasma volume expansion in early pregnancy, hemodynamic alterations, and lower cardiac output have been postulated as a cause of fetal growth restriction (17, 18, 20, 58, 60, 63). Thus, failure or perturbations of normal vascular adaptations may be a cause of poor pregnancy outcomes such as fetal growth restriction.

Smoking during pregnancy has been associated with reduced vascularization of the placenta, and three-dimensional Doppler ultrasound measures flow at 11–14 wk of gestation without impacting placental volume (57). Smoking has also been associated with impaired flow-mediated dilation in pregnant women, similar to previous reports in nonpregnant subjects (54). Increases in fetal and maternal heart rate are seen immediately following smoking (52). While smoking has been demonstrated to increase resistance in the uterine and umbilical arteries, no change in uterine blood flow was detected (3, 7, 30, 31, 48, 52), or if a change was observed, uterine blood flow...
Maternal cigarette smoke exposure could impair the normal vascular adaptations to pregnancy; however, the mechanism that causes fetal growth restriction is not well defined. The primary objective of this study was to determine whether maternal vascular adaptation to pregnancy was affected by smoke exposure in an animal model with cigarette smoke-induced fetal growth restriction. Use of an animal model of inhaled cigarette smoke during pregnancy is crucial to examining mechanisms for smoking-related pregnancy complications as well as testing potential interventional strategies.

**METHODS**

C57Bl/6J adult mice (n = 23; Jackson Laboratories, Bar Harbor, ME) were maintained on a 12:12-h light-dark cycle, with food (breeder chow) and water available ad libitum. The C57Bl mouse has been reported to be more susceptible than other mouse strains to cigarette smoke when lung inflammation and oxidative end points were measured, so this strain was selected for our studies (73). Two female and one male mouse were caged together for natural breeding. The morning of a positive vaginal plug was considered to be day 0 of gestation (n = 11). On day 4 of gestation, pregnant dams (n = 6) as well as virgins (n = 6) were exposed to whole body sidestream smoke in an inhalation chamber (model TE-10, Teague Enterprises, Davis, CA) for 4 h, with smoke exposure increased gradually over the first 5 days from 30 mg/m³ total suspended particles (TSP) to 100 mg/m³ TSP (average 67 mg/m³) and maintained at 100 mg/m³ TSP through day 16–17 of pregnancy (late gestation). TSP matter was monitored hourly using a particle impacter. The average TSP reading for the exposure was 63 mg/m³, which is representative of moderate-to-heavy smoking in humans. Mice were weighed throughout pregnancy, and percent weight gain from the initial weight to day 17 of pregnancy was calculated. Additionally, animals were monitored throughout the exposure period for signs of morbidity (weight loss and lack of activity or grooming). No animal exhibited signs of severe stress, and no experiments had to be discontinued. Control animals (n = 6, virgins and n = 5 pregnant controls) were placed in an adjacent chamber for the same amount of time as the exposed mice, without smoke exposure, to control for stress related to the noise generated from the smoke inhalation chamber. Smoke exposure was determined in EDTA plasma samples by measurement of cotinine levels by a Direct ELISA kit (Immunalysis, Pomona, CA). The sensitivity of the kit is 1 ng/ml. Plasma samples were obtained 18–19 h after smoke exposure. The Magee-Womens Research Institute Animal Care and Use Committee reviewed and approved this protocol.

**Isolated arteriograph experiments.** Mice were killed with an intraperitoneal injection of pentobarbital sodium (Nembutal), and the arteries were immediately removed from virgin or day 17 or 18 pregnant mice (with the exception of 1 animal at gestational day 16) and placed in cold HEPES-buffered physiological saline solution at pH 7.4 (HPSS). HPSS contained (mmol/l) 142 sodium chloride, 4.7 potassium chloride, 1.17 magnesium sulfate, 2.5 calcium chloride, 1.18 potassium phosphate, 10 HEPES, and 5.5 dextrose. Second-order mesenteric arteries, segments of the main uterine artery and renal arteries, were isolated and cleaned of fat and connective tissue. Isolated arteries with use of a pressurized arteriograph. An approach modified from MacPherson et al. was utilized in these experiments (21, 37). Arteries with intraluminal pressure stabilized at 60 mmHg were subjected to a rapid increase in pressure to 80 mmHg. This manipulation was performed in triplicate, with 4- to 6-min intervals between pressure steps. Arteries studied in this manner have a beginning steady-state diameter at 60 mmHg (D₁) followed by a rapid increase in diameter with the pressure step and then an active constriction to a final diameter (D₂) at 80 mmHg. The pressure-induced tone is the percent change in diameter from D₁ to D₂.

**Agonist concentration response.** Arteries were first preconstricted with phenylephrine to ~50% of the baseline diameter at 60 mmHg. Preconstricted arteries were exposed to cumulative concentrations of the endothelium-dependent vasodilator methacholine (5 × 10⁻⁹–10⁻⁴ mol/l). Cumulative concentration-response curves to methacholine were determined. The arteries were then rinsed with HPSS. Arteries were reconstricted with phenylephrine and exposed to cumulative concentrations of the nitric oxide (NO) donor sodium nitroprusside (10⁻⁶–10⁻⁴ mol/l) to assess non-endothelium-dependent vasodilatory capacity.

Arterial smooth muscle was inactivated by treatment with a calcium-free HPSS in combination with 1 × 10⁻⁴ mol/l papaverine and 1 × 10⁻⁴ mol/l EGTA in calcium-free HPSS for ≥10 min; then passive luminal diameter and wall thickness were measured at 0–150 mmHg.

**Calculations.** Passive mechanical properties of arteries were assessed using pressure-diameter relationships for distensibility and stress-strain calculations. Distensibility is defined as the relative change in diameter per unit change in pressure in arteries with inactivated smooth muscle. To obtain the relative change in diameter, the diameter measured at each pressure was normalized to an initial diameter of 5 mmHg. The slopes of the linear portion (5–100 mmHg) of the pressure-diameter curves were used to compare distensibilities between groups. The circumferential stress-strain relationship was calculated to further describe the passive mechanical properties of the arteries. This parameter was normalized for wall thickness and characterized the stiffness of the vascular wall. Circumferential stress describes the force exerted on the vascular wall per unit of tissue and is derived from the following equation: stress = (P × D)/2T, where P is transmural pressure in millinewtons per square millimeter (1 mmHg = 0.133 mN/mm²), D is diameter, and T is wall thickness. Circumferential strain represents the response of an artery to the force or intraluminal pressure it experiences. Strain was calculated as follows:

\[
\text{Strain} = \frac{\text{Diameter at 60 mmHg}}{\text{Diameter at baseline}} \times 100
\]

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>Uterine Arteries</th>
<th>Mesenteric Arteries</th>
<th>Renal Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin Control</td>
<td>6</td>
<td>166 ± 22</td>
<td>225 ± 9</td>
<td>196 ± 10†</td>
</tr>
<tr>
<td>Smoke-exposed</td>
<td>6</td>
<td>199 ± 12</td>
<td>231 ± 15</td>
<td>130 ± 19</td>
</tr>
<tr>
<td>Pregnant Control</td>
<td>5</td>
<td>320 ± 17*†</td>
<td>246 ± 8</td>
<td>183 ± 20</td>
</tr>
<tr>
<td>Smoke-exposed</td>
<td>6</td>
<td>328 ± 26†</td>
<td>232 ± 10</td>
<td>156 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. control virgin; †P < 0.05 vs. smoke virgin (by 2-way ANOVA within a vascular bed).
\( (D_f - D_0)/D_0 \), where \( D_0 \) is the initial diameter at 5 mmHg and \( D_f \) is the diameter at the new pressure. Stress was calculated for specified strains and compared between groups.

**Statistical analysis.** Data are expressed as means ± SE. Student’s t-test was used for all pairwise comparisons of parametric data. All data were first analyzed by one- or two-factor ANOVA. If significant main effects or interactions were observed, then individual group means were compared, with the level of significance for each test adjusted by Bonferroni’s method to account for multiple comparisons or by orthogonal contrasts. Two-way repeated-measures ANOVA was

### Table 2. Pregnancy data

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Litter Size</th>
<th>Pup Wt, g</th>
<th>Placental Wt, g</th>
<th>Fetoplacental Ratio</th>
<th>% Maternal Wt Gain During Pregnancy</th>
<th>Gestational Age, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control pregnant</td>
<td>5</td>
<td>6.0 ± 1.3</td>
<td>1.0 ± 0.1*</td>
<td>0.1 ± 0.01</td>
<td>8.9 ± 0.7*</td>
<td>37.1 ± 4.0</td>
<td>17.6 ± 0.2 (17–18)</td>
</tr>
<tr>
<td>Smoke pregnant</td>
<td>6</td>
<td>6.8 ± 0.6</td>
<td>0.9 ± 0.1</td>
<td>0.1 ± 0.01</td>
<td>7.1 ± 1.1</td>
<td>43.8 ± 2.2</td>
<td>17.5 ± 0.3 (16–18)</td>
</tr>
</tbody>
</table>

Values are means ± SE, with range in parentheses. *P < 0.05 (by Student’s t-test).
used to compare changes in diameter at different doses between groups. \( P < 0.05 \) represented statistical significance.

RESULTS

Pregnancy-associated maternal vascular remodeling as assessed by isolated arterial diameter at 60 mmHg was seen only in the uterine vasculature, where diameters increased in both control and smoke-exposed arteries from pregnant mice (Table 1). Renal arteries isolated from smoke-exposed virgin mice were significantly smaller than those isolated from virgin controls (Table 1). Maternal weight gain during pregnancy was not significantly different between smoke-exposed mothers and controls (44% vs. 37% increase in weight during pregnancy, \( P = 0.158 \); Table 2). Fetal weights were significantly reduced in smoke-exposed compared with control fetuses (0.88 ± 0.1 vs. 1.0 ± 0.08 g, \( P = 0.02 \)), while litter sizes were not different (Table 2). Fetoplacental ratios were significantly reduced in smoke-exposed mothers due to the >10% reduction in fetal weights (Table 2). The levels of the nicotine metabolite cotinine in smoking mice were significantly elevated at 18–19 h after exposure to 4 h of cigarette smoke compared with control mice [5.9 ± 9.2 vs. 4.2 ± 0.8 (virgin) and 33.2 ± 21 vs. 2.8 ± 0.4 ng/ml (pregnant)]. Cotinine levels >50 ng/ml are typically associated with active smokers (61).

Relaxation responses. Isolated arteries were preconstricted with phenylephrine to ~50% of their initial diameter. There was no significant difference in phenylephrine responses between the groups. Maximal endothelium-mediated relaxation responses to methacholine were significantly impaired in both the uterine (3.4 ± 3% constriction remaining in control pregnant vs. 26.3 ± 11% in smoke-exposed pregnant mice) and mesenteric (9.1 ± 4% constriction remaining in control pregnant vs. 44.1 ± 12% in smoke-exposed pregnant mice) vasculature of pregnant mice exposed to cigarette smoke during gestation (Fig. 1, A and B, Table 3). No difference in endothelium-mediated relaxation was seen in renal arteries isolated from pregnant mice exposed to cigarette smoke (Fig. 1C); however, relaxation was significantly reduced in renal arteries from virgin mice exposed to smoke (109.9 ± 35% constriction remaining in smoke-exposed virgin vs. 8.9 ± 19% in control virgin mice; Fig. 1F, Table 3). In contrast, relaxation responses to methacholine were unchanged in mesenteric arteries from virgin mice (Fig. 1E), and uterine arteries from smoke-exposed virgin mice were more sensitive than those from controls (Fig. 1D). The \( EC_{50} \) for methacholine was not significantly different in any of the vascular beds.

Smoke exposure did not change endothelium-independent relaxation responses to the NO donor sodium nitroprusside in either vessel bed (mesenteric and uterine). There was a significant increase in sensitivity to NO in arteries from pregnant compared with virgin mice, in both control and smoke-exposed mesenteric arteries and among smoke-exposed uterine arteries (Table 3).

Pressure stimulated arterial tone. Myogenic reactivity was assessed in isolated arterial segments using repeated steps in pressure from 60 to 80 mmHg. The percent change in diameter of uterine and mesenteric arteries was not significantly different between the groups (Fig. 2, A and B). The percent change in diameter of renal arteries isolated from control pregnant mice was significantly greater (i.e., less pressure-induced reactivity) than that of arteries isolated from virgin mice, indicative of less myogenic tone, while the diameter of renal arteries

### Table 3. Summary arterial data

<table>
<thead>
<tr>
<th>Artery</th>
<th>Virgin</th>
<th>Smoke-exposed</th>
<th>Virgin</th>
<th>Smoke-exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>15.9 ± 10.7</td>
<td>11.4 ± 0.9</td>
<td>9.1 ± 20.7</td>
<td>6.8 ± 2.9</td>
</tr>
<tr>
<td>NO relaxation</td>
<td>% constriction</td>
<td>% constriction</td>
<td>% constriction</td>
<td>% constriction</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>0.19 ± 0.06</td>
<td>0.06 ± 0.009</td>
<td>0.29 ± 0.18</td>
<td>0.61 ± 0.20</td>
</tr>
<tr>
<td>( EC_{50} ) sodium nitroprusside</td>
<td>0.40 ± 0.17</td>
<td>1.6 ± 0.11</td>
<td>0.28 ± 18</td>
<td>0.08 ± 0.18</td>
</tr>
<tr>
<td>Myogenic tone</td>
<td>% change in diameter</td>
<td>% change in diameter</td>
<td>% change in diameter</td>
<td>% change in diameter</td>
</tr>
<tr>
<td>Stress, 10 dyn/cm²</td>
<td>5.5 ± 7.1</td>
<td>3.2 ± 7.7</td>
<td>2.2 ± 3.7</td>
<td>1.0 ± 3.2</td>
</tr>
<tr>
<td>Stress, 20 dyn/cm²</td>
<td>2.7 ± 0.9</td>
<td>15.4 ± 0.8</td>
<td>4.9 ± 0.8</td>
<td>4.9 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Endothelial relaxation is expressed as %phenylephrine constriction remaining at maximum dose of methacholine. Nitric oxide (NO) relaxation is %phenylephrine constriction remaining at maximum dose of sodium nitroprusside. Myogenic tone is %phenylephrine constriction remaining at steady-state tension at 10 mmol/L phenylephrine. Myogenic tone at 20 dyn/cm² is calculated stress in the artery in response to a step in intimal blood pressure from 60 to 80 mmHg. Positive values indicate increased reactivity compared with control fetuses. NA, not assessed.

\( P < 0.05 \) vs. control pregnant; \( P < 0.05 \) vs. control control; \( P < 0.05 \) vs. control pregnant; \( P < 0.05 \) vs. control control.
from smoke-exposed pregnant mice was not changed from that of arteries from either control or smoke-exposed virgin mice (Fig. 2C).

**Passive mechanical properties of isolated arteries.** The distensibility of isolated uterine arteries, demonstrated by percent change in diameter plotted against change in pressure, is shown in Fig. 3A. There was no significant difference in distensibility of isolated uterine or mesenteric arteries from any of the study groups as determined by an analysis of the pressure vs. percent change in diameter over the linear portion of the pressure-diameter curve (10–60 mmHg). There was a significant difference in wall thickness only at low pressures in uterine arteries from smoke-exposed pregnant mice compared with virgin controls (33 ± 9 vs. 22 ± 2 μm at 20 mmHg, P < 0.05); comparison of control pregnant mice and virgin controls showed a similar trend (33 ± 5 vs. 26 ± 2 μm at 20 mmHg), but the difference was not significant.

There was no significant difference in stress-strain characteristics of uterine arteries from smoking and control virgin mice (Fig. 3B, Table 3). There was a greater stress associated with a given strain in uterine arteries from pregnant mice than in arteries from virgin mice, with the greatest pregnancy-associated change in the control mice (Fig. 3B). Thus, while the modulus of elasticity (or stiffness) was higher in uterine vessels from control pregnant mice than control virgin mice, stiffness was decreased in vessels from smoke-exposed pregnant mice compared with control pregnant mice.

**DISCUSSION**

This study used a pregnant mouse model of passive cigarette smoke exposure at levels causing reduced fetal weight to determine if this exposure would adversely affect the maternal vascular adaptation to pregnancy. Smoke exposure beginning on day 4 of pregnancy did not affect the litter size or maternal weight gain during pregnancy. Cigarette smoke exposure during days 4–17 of pregnancy in mice caused a reduction in the fetal-to-placental ratio due to a low fetal weight relative to placental weight. Disproportionate fetal-to-placental growth has previously been reported in humans (31, 70, 72) and in mice (19). A >10% reduction in fetal weight is consistent with previously published data in rats and mice based on the dose and time frame of exposure (19, 23, 36). In mice, mainstream or sidestream (passive) exposure to cigarette smoke prior to day 6 of gestation has been reported to decrease fetal weight and crown-rump length in C57Bl mice (19). The reduction in fetal weight independent of placental weight reduction has been postulated to result from an abnormal placenta with impaired function and/or fetal/placental exposure to toxicants present in smoke (9).

**Arterial function and structure measures after smoke exposure.** Three maternal vascular beds were examined in the current study. Endothelium-mediated relaxation was impaired in mouse mesenteric and uterine vessels exposed to cigarette smoke during pregnancy and in the renal bed of virgin mice. Impaired maternal vascular function of the endothelium has also recently been...
in response to subcutaneous injection of polycyclic aromatic hydrocarbon acting through the aryl hydrocarbon receptor (16).

Component of cigarette smoke with potential to impact vascular function. The main constituents of environmental tobacco smoke are very similar to those of inhaled cigarette smoke, including tar, nicotine, carbon monoxide, cadmium, and aromatic hydrocarbons. Nicotine alone has previously been shown to reduce uterine blood flow in pregnant sheep and rats (8, 11, 42, 43), mediated through release of epinephrine. However, some reports have shown no significant fetal growth restriction with nicotine or high levels of epinephrine (8, 29, 64). A limitation of these studies was the route of nicotine dosing (via oral gavages, implantable osmotic minipump, subcutaneous intravenous injections, or parenteral bolus); these routes require high nicotine doses to achieve relevant plasma concentrations for heavy smokers that also cause maternal cardiovascular effects in rodents. Nicotine does not reproduce all the effects seen with cigarette smoke, leading us and others to believe that nicotine alone cannot cause the acute endothelial toxicity related to passive smoking (6) and that other constituents of cigarette smoke during pregnancy likely play a role in fetal growth restriction (29). It is important to note that the vascular effects reported here were observed 18–19 h after the last smoke exposure. Nicotine has a short half-life of 5–8 min in C57Bl mice, so the effects are not likely to be the direct effect of nicotine in the maternal circulation (51, 62).

The inhalation chamber permits a route of delivery and dosing levels of cigarette smoke relevant in humans. It has been shown in vitro that lipid-soluble smoke particles reduce endothelium-dependent relaxation, resulting in endothelial dysfunction in rat mesenteric vessels and human middle cerebral arteries (74). DMSO-soluble particles extracted from smoke are toxic to arterial endothelial and smooth muscle cells in culture. These particles are transported by lipoproteins to the arterial wall, directly impairing function of cells via modification of lipoprotein properties (55, 74).

This is the first study that investigates the myogenic response of vessels from vascular beds of pregnant mice exposed to cigarette smoke. Our study demonstrates that, like rats, there is a significant reduction in pressure-change-induced vascular tone in renal vessels from pregnant compared with virgin mice, and smoking during pregnancy eliminates the pregnancy-associated loss of tone (21). Reduced myogenic tone has also been observed in the small mesenteric and main uterine arteries of pregnant mice (67) and in the mesenteric and renal arteries of pregnant rats (21, 35) and can be attributed to the increased endothelium-derived NO during pregnancy. The pattern of affected vascular beds in the current study may be influenced by the time frame of pregnancy-initiated changes relative to smoke exposure. Early adaptations, including renal changes and the initiation of uterine artery remodeling, would have occurred prior to smoke exposure on day 4 of gestation.

Mechanical properties of vessel walls. Murine uterine arteries remodel during pregnancy by increasing wall mass early in pregnancy and then increasing intraluminal diameter with no change in densities of elastin and collagen in the extracellular matrix (66). Previous studies showed increased distensibility of uterine arteries of pregnant sheep and guinea pigs and uteroplacental arteries of rats during pregnancy (25, 38, 46), but arteries from pregnant sheep were stiffer than those from virgin sheep (25), while stiffness did not change in the guinea pig (38). This is demonstrated through calculations of flow in the brachial artery of smoking women at 28–32 wk of gestation in response to reactive hyperemia (54). Our data indicate that specific maternal arterial beds may be more susceptible to cigarette smoke exposure during pregnancy, and work is ongoing to begin to elucidate the mechanisms of the endothelial dysfunction.

Arterial diameter was increased only within the uterine vascular bed compared with arteries from the same vascular bed isolated from virgin mice. The dramatic expansion of arterial diameter within the uterus encompasses the main uterine artery as well as the arteries supplying the implantation site. Gokina et al. (24) showed that the most profound changes occur in the arteries directly supplying the implantation site in the uterus of rodents, while vasodilatation is less profound in ancillary arteries and veins. A 40–50% increase in main uterine artery diameter is expected during a mouse pregnancy (65, 66). In the mouse, arterial fetoplacental vasculature (measured as arterial surface area and volume) is unchanged between days 13.5 and 13.5 of gestation but more than doubles from day 15.5 to 18.5 (56). Changes in the fetoplacental structure and fetal growth restriction have been demonstrated...
the first study to show the effect of smoking on the stress-strain relationship of uterine vessels in pregnant mice. There is a significant decrease in the modulus of elasticity (or stiffness) of uterine vessels when pregnant mice are exposed to cigarette smoke; i.e., the vessels become less stiff. In this case, smoking affects the natural adaptations to pregnancy by not enabling the vessels to attain the desired level of stiffness required to accommodate the large increase in blood flow. We found no significant changes in stress-strain relationships of mesenteric and renal vessels of smoking and nonsmoking pregnant mice.

The pathogenesis of the relationship between smoking and growth restriction remains unclear. Potential mechanisms for poor fetal growth include direct effects of nicotine on uterine and umbilical vasculature resulting in vasoconstriction of the uteroplacental circulation (71), increased carboxyhemoglobin in the maternal circulation leading to chronic oxygenation insufficiency of the fetus (59), and chronic exposure of the fetoplacental unit to the thousands of secondary components of tobacco smoke or their metabolites. Smoke exposure results in smaller fetuses in pregnant mice, with significant impairment of the maternal uterine and mesenteric relaxation capacity and renal vascular myogenic tone.

Perspectives and Significance

While smoking is well recognized as a modifiable risk factor associated with numerous pregnancy complications, ~10% of women will continue to smoke during pregnancy. The uteroplacental vasculature is a likely target of cigarette smoke in both humans and rodent models. The present study and the work of Quinton et al. (54) extend this information to other maternal vascular beds in mice and humans. Our work also demonstrates that sensitivity to smoke exposure during pregnancy is dependent on the vascular bed examined. The data obtained using a smoke inhalation exposure model in mice and detailed studies of the uteroplacental vascular changes of pregnancy (2, 47, 56) indicate that this is an ideal model system for mechanistic studies as well as for elucidation of both critical time points and components of smoke.

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


