Maternal tobacco use is associated with increased markers of oxidative stress in the placenta

Elena Sbrana, PhD; Melissa A. Suter, PhD; Adi R. Abramovici, MD; Hal K. Hawkins, MD, PhD; Joan E. Moss, RN, MSN; Lauren Patterson, MD; Cynthia Shope, MS; Kjersti Aagaard-Tillery, MD, PhD

OBJECTIVE: We sought to extend our prior observations and histopathologically characterize key metabolic enzymes (*CYP1A1*) with markers of oxidative damage in the placental sections from smokers.

STUDY DESIGN: Placental specimens were collected from term singleton deliveries from smokers (n = 10) and nonsmokers (n = 10) and subjected to a detailed histopathological examination. To quantify the extent of oxidative damage, masked score-graded (0-6) histopathology against 4-hydroxy-2-nonenal (4-HNE) and 8-hydroxydeoxyguanisine (8-OHdG) was performed. Minimal significance ($P < .05$) was determined with a Fisher's exact and a 2-tailed Student *t* test as appropriate.

RESULTS: We observed a significant increase in the presence of syncytial knots in placentas from smokers (70% vs 10%, $P = .02$). These gross observations were accompanied by a significant aberrant placental aromatic hydrocarbon metabolism (increased CYP1A1, 4.4 vs 2.1, $P = .002$) in addition to evidence of oxidative damage (4-HNE 3.4 vs 1.1, $P = .00005$; 8-OHdG 4.9 vs 3.1, $P = .0038$).

CONCLUSION: We observed a strong association between maternal tobacco use and aberrant placental metabolism, syncytial knot formation, and multiple markers of oxidative damage.

Key words: immunohistochemistry, intrauterine growth retardation, maternal smoking, metabolic stress, oxidative stress, placenta

Cite this article as: Sbrana E, Suter MA, Abramovici AR, et al. Maternal tobacco use is associated with increased markers of oxidative stress in the placenta. Am J Obstet Gynecol 2011;205:246.e1-7.

Although the concerning effects of maternal tobacco smoke on fetal growth have been well reported for more than 3 decades, it remains today one of the leading preventable causes of fetal growth restriction in developed and developing countries.[1-4] In the seminal report from Simpson,[5] it was reported that mothers who smoked 10 cigarettes or more per day delivered infants with a decrease in birthweight of approximately 200 g compared with neonates from nonsmoking mothers. However, not all fetuses exposed to maternal tobacco smoke are growth restricted.[1,2,6,7] Susceptibility to tobacco exposure likely involves several factors including, but not limited to, epidemiological, genetic, epigenetic, and socioeconomic.[1,2]

Nicotine, a principal alkaloid of tobacco smoke, has been shown to mediate constriction of the intrauterine vessels and result in increased proliferation of placental syncytiotrophoblasts.[8] Potentially harmful deoxyribonucleic acid (DNA) adducts (metabolic products of polycyclic aromatic hydrocarbons [PAH]) are known to cross or collect in the placenta of smokers.[9,10]

PAH compounds, together with nitrosamines, comprise likely carcinogenic species in tobacco smoke.[11,12] The majority of chemical carcinogens are metabolized in a sequential series of 2-phase enzymatic metabolic reactions (Figure 1).[1,2] Phase I enzymes such as *CYP1A1* metabolically activate PAH compounds into oxidized derivatives, resulting in reactive oxygen intermediates capable of covalently binding DNA to form adducts.[13] In turn, these reactive electrophilic intermediates can be detoxified by phase II enzymes, such as the glutathione *S*-transferase (GSTT1), via conjugation with endogenous species to form hydrophilic glutathione conjugates, which are then readily excreted.[13] Thus, the coordinated expression of these enzymes and their relative balance may determine the extent of cellular DNA damage and related development of adverse outcomes.

We have previously demonstrated that in a large matched cohort, deletion of fetal *GSTT1* (a phase II pathway gene; Figure 1) is associated with birthweight reduction in pregnancies exposed to maternal tobacco use.[6] We have also shown that increased placental *CYP1A1* expression was specifically and significantly associated with hypomethylation of XRE-proximal CpG dinucleotides in the *CYP1A1* promoter region in smokers compared with nonsmokers.[14] An in-

From the Departments of Pathology (Drs Sbrana and Hawkins), and Obstetrics and Gynecology (Ms Moss), University of Texas Medical Branch, Galveston, and the Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston (Drs Suter, Abramovici, Patterson, and Aagaard-Tillery and Ms Shope), TX.

Presented at the 31st Annual Meeting of the Society for Maternal-Fetal Medicine, San Francisco, CA, Feb. 7-12, 2011.

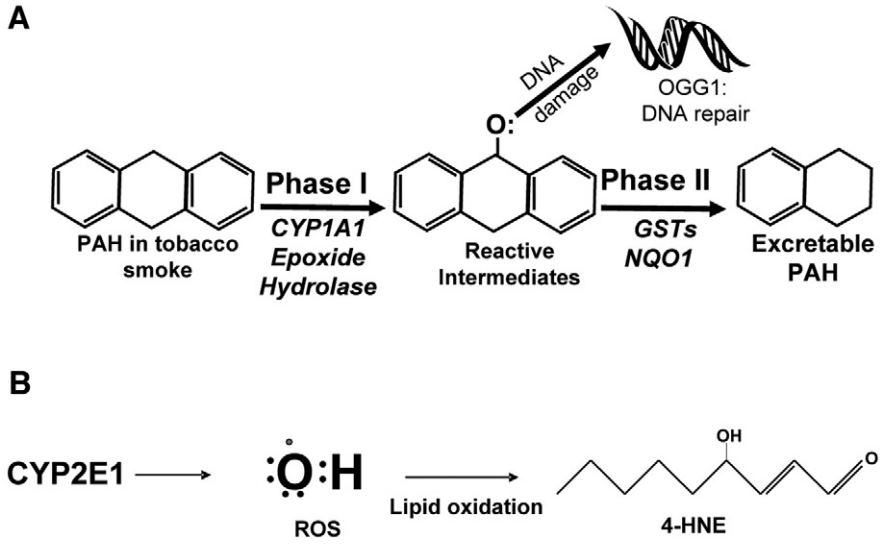
Received March 11, 2011; revised April 27, 2011; accepted June 7, 2011.

Reprints: Kjersti Aagaard-Tillery, MD, PhD, Baylor College of Medicine, Division of Maternal-Fetal Medicine, 1 Baylor Plaza, Jones 314, Houston, TX 77030. aagaardt@bcm.edu.

This study was supported in part by the National Institutes of Health Director New Innovator Award (DP2120OD001500-01) and National Institutes of Child Health and Human Development/National Institutes of Diabetes and Digestive and Kidney Diseases Grant R01DK080558-01 (both to K.A.-T.) and National Institutes of Health Grant REACH IRACDA K12 GM084897 (to M.A.S.).

0002-9378/$36.00 • © 2011 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2011.06.023

FIGURE 1
Processing of xenobiotics in the placenta



A, Polycyclic aromatic hydrocarbons are processed in a 2-step process. An increase in the phase I enzymes is reported in the placenta in mothers who smoke compared with nonsmoking controls. An increase in phase I enzymes metabolizes PAHs into ROS, which can lead to oxidative DNA damage, such as 8-OHdG. **B,** Processing of xenobiotics by the phase I enzyme CYP2E1 creates ROS, which can lead to oxidative lipid damage such as 4-HNE.

4-HNE, 4-hydroxy-2-nonenal; *8-OHdG*, 8-hydroxydeoxyguanisine; *PAHs*, polycyclic aromatic hydrocarbons; *ROS*, reactive oxygen species.

Sbrana. Placental oxidative stress with maternal smoking. Am J Obstet Gynecol 2011.

crease in phase I enzymes without a compensatory increase in phase II enzymes has the potential to create reactive species within the cell. These unprocessed reactive oxygen species (ROSs) have the unmitigated potential to lead to DNA-adduct–mediated damage and lipid oxidation, perpetuating the cycle of modulated cellular and molecular physiology (Figure 1).

In this study, we hypothesized the disrupted metabolic pathways converge at the cellular level to increase markers of oxidative stress in the placenta. To quantify the extent of DNA damage and oxidative damage, we used 2 well-characterized markers: 8-hydroxydeoxyguanisine (8-OHdG; a marker of DNA damage) and 4-hydroxy-2-nonenal (4-HNE; a marker for oxidative lipid damage) as determinates of cellular oxidative stress.[15,16] We therefore sought to extend our prior observations and histopathologically characterize key metabolic enzymes (*CYP1A1*) with markers of oxidative damage in placental sections from smokers.

TABLE 1
Characteristics of the study population

Characteristic	Nonsmokers (n = 10)	Smokers (n = 10)	*P* value
Maternal age, y	29.7 ± 1.8	27.8 ± 2.1	.504
Maternal BMI, kg/m^2	26.5 ± 0.9	22.7 ± 3.1	.251
Gestational age, wks	39.3 ± 0.7	39.0 ± 0.4	.719
Infant weight, g	3619 ± 128	3159 ± 144	.029[a]
Infant length, cm	49.8 ± 0.7	48.2 ± 0.9	.226

[a] In our nested cohort design, after matching for maternal characteristics and gestational age, we observed a statistically significant association between infant birth weight and maternal smoking.

Sbrana. Placental oxidative stress with maternal smoking. Am J Obstet Gynecol 2011.

Materials and Methods

Study population

Placental samples (n = 20) for this study were obtained from subjects selected from a well-described cohort of 20 self-reported smokers in addition to 53 nonsmoking controls; this has been previously validated as an accurate measure of maternal tobacco exposure.[17]

The Institutional Review Board of Baylor College of Medicine and its affiliated institutions approved this study, and written informed consent was obtained from each participant at the time of enrollment. Data collected from each patient included age, ethnicity, height and weight, past obstetrical history, gestational age at delivery, and potential maternal comorbidities. Data collected from the newborns included sex, Apgar scores, weight and length, and level of resuscitation interventions if any.

Exclusion criteria included multiple gestations; known fetal anomalies; and maternal hepatic, hypertensive, or endocrine disorders. For the analysis reported herein, subjects were matched in a nested cohort design by virtue of maternal age (±3 years), race/ethnicity, body mass index (BMI), and gestational age (±1 week). Consistent with a nested cohort design, matching was performed prior to knowledge of the primary outcomes (ie, histopathology and immunohistochemistry) and without consideration of fetal factors (beyond gestational age) including fetal weight or length or neonatal outcome. In such a manner, an initial 20 matched subjects were analyzed with minimized potential for selection bias. This is as noted in Table 1.

Collection and standardized processing of placental samples

Placental specimens were collected immediately after delivery, systematically stored, and processed for histopathology within 12 hours. Standardized collection and section methodology included uniform triplicate 3 cm excisional blocks at a prescribed 4 cm trinary distance from the umbilical cord insertion along with a section from the insertion point and ran-