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REVIEW

Long-Term Consequences of Fetal and Neonatal Nicotine Exposure: A Critical Review

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Cigarette smoking during pregnancy is associated with numerous obstetrical, fetal, and developmental complications, as well as an increased risk of adverse health consequences in the adult offspring. Nicotine replacement therapy (NRT) has been developed as a pharmacotherapy for smoking cessation and is considered to be a safer alternative for women to smoking during pregnancy. The safety of NRT use during pregnancy has been evaluated in a limited number of short-term human trials, but there is currently no information on the long-term effects of developmental nicotine exposure in humans. However, animal studies suggest that nicotine alone may be a key chemical responsible for many of the long-term effects associated with maternal cigarette smoking on the offspring, such as impaired fertility, type 2 diabetes, obesity, hypertension, neurobehavioral defects, and respiratory dysfunction. This review will examine the long-term effects of fetal and neonatal nicotine exposure on postnatal health.

Key Words: **nicotine; pregnancy; fetal programming of adult disease; smoking cessation.**

It has been well documented that cigarette smoking during pregnancy is associated with a number of adverse obstetrical outcomes including: spontaneous abortion (George *et al.*, 2006), placenta previa (Chelmow *et al.*, 1996; Faiz and Ananth, 2003; Hung *et al.*, 2007), placental abruption (Ananth *et al.*, 1999), preterm birth (Fantuzzi *et al.*, 2007; Kolas *et al.*, 2000), stillbirth (Hogberg and Cnattingius, 2007; Wisborg *et al.*, 2001), fetal growth restriction (Hammoud *et al.*, 2005; Nordentoft *et al.*, 1996), low birth weight (Bernstein *et al.*, 2005; Jaddoe *et al.*, 2008), and sudden infant death syndrome (SIDS) (Mitchell and Milerad, 2006). Smoking cessation or at least reduction of cigarette smoking during pregnancy can ameliorate damage to the developing fetus (Lindley *et al.*, 2000; Pickett *et al.*, 2003). Indeed, smoking cessation programs based on behavioral therapy, which are implemented during pregnancy, have been shown to reduce the incidence of low birth weight and preterm birth (Lumley *et al.*, 2004).

Although cigarette smoking during pregnancy is associated with adverse fetal, obstetrical, and developmental outcomes, 15–20% of all women smoke throughout the duration of pregnancy (Andres and Day, 2000; Bergmann *et al.*, 2003), despite intentions to refrain from smoking during that period (Okuyemi *et al.*, 2000). Approximately 75% of pregnant smokers report the desire to quit smoking (Ruggiero *et al.*, 2000), but only 20–30% successfully abstain from smoking during pregnancy and half of these women relapse within 6 months of parturition (Ebert and Fahy, 2007). Smoking cessation is most effective if implemented before pregnancy or prior to the initiation of prenatal care (Tong *et al.*, 2008). For most women, nicotine dependence is a significant element of their smoking behavior (Okuyemi *et al.*, 2000), and the highly addictive nature of nicotine makes smoking cessation difficult. Indeed, the majority of adverse physiological symptoms associated with smoking cessation (cravings, irritability, restlessness, anxiety, and increased appetite) have been attributed to nicotine withdrawal (Glynn *et al.*, 2009). Therefore, nicotine replacement therapy (NRT) has been widely developed as a pharmacotherapy of smoking cessation and is considered to be of benefit for pregnant women who are highly dependent and have been unable to quit smoking by other means (Benowitz and Dempsey, 2004; Okuyemi *et al.*, 2000; Ontario Medical Association [OMA], 2008; Peters and Morgan, 2002).

In pregnant women who smoke or use NRT, nicotine crosses the placenta, concentrates in fetal blood and amniotic fluid, and is detectable in breast milk during lactation (Jordanov, 1990; Lambers and Clark, 1996; Luck and Nau, 1987). Indeed, during the first week of life, urinary cotinine levels in infants of smoking mothers who are exclusively breast-fed are significantly higher than those who are only bottle fed (fully breast-fed median 801 ng cotinine/mg creatinine [range 325–1693] vs. fully bottle fed median 65 ng cotinine/mg creatinine [range 28–101]; Schwartz-Bickenbach *et al.*, 1987). Therefore, maternal NRT use results in both fetal and neonatal exposure to nicotine.

NRT in the form of gum, nasal spray, and lozenges is currently classified as a Pregnancy Category C drug, whereas the transdermal nicotine patch is classified in pregnancy as Category D (Benowitz and Dempsey, 2004; Pauly and Slotkin, 2008). Based on these U.S. Food and Drug Administration classifications, it is generally agreed that the risk to the fetus of continued smoking outweighs any potential adverse effects of NRT (Glynn *et al*., 2009; OMA, 2008). Furthermore, NRT is thought of as a safer alternative to smoking during pregnancy because the mother and fetus are exposed to one chemical instead of the thousands of chemicals found in cigarette smoke (Glynn *et al*., 2009; OMA, 2008). It has also been argued that because NRT usually delivers a dose of nicotine that is equivalent to smoking 10 cigarettes a day, the fetus will be exposed to much less nicotine than children born to women who smoke heavily during pregnancy (Oncken and Kranzler, 2003). The OMA currently recommends that NRT should be made available to pregnant women who are unable to quit smoking using nonpharmacologic means (OMA, 2008). In addition, the Committee on Safety of Medicines (CSM) and Medicines and Healthcare Regulatory Authority (MHRA) in the United Kingdom recently changed their policy to recommend NRT to pregnant and breastfeeding mothers, stating that although there is a theoretical risk that nicotine could cause harmful effects, in practice, none have been found to date (Action on Smoking and Health, 2005). However, there are several issues with the OMA and CSM/MHRA recommendations. First, NRT use compared with placebo does not appear to increase the probability of successful smoking cessation during pregnancy. Although NRT is highly effective for smoking cessation in non–pregnant smokers (reviewed in Glynn *et al*., 2009), there is currently no evidence to suggest that NRT use is effective for smoking cessation in pregnant women (assessed by meta-analysis in Lumley *et al*., 2004). These findings may be attributed to the increased rate of nicotine metabolism in pregnant versus non–pregnant smokers (60% higher nicotine clearance and 140% higher cotinine clearance during pregnancy; Dempsey *et al*., 2002) or may relate to the low adherence to NRT among pregnant smokers (Fish *et al*., 2009). Despite the lack of evidence to support the efficacy of NRT use during pregnancy, the percentage of pregnancies in which NRT was prescribed has increased steadily between 1998 and 2004 (Coleman, 2008). Second, nicotine may not be the "safe" chemical in cigarettes as was previously assumed. Notably, in their recently updated smoking cessation document, the OMA has acknowledged that there is no safe dose of nicotine during pregnancy (OMA, 2008).

Ginzel *et al*. (2007) have recently raised concerns about NRT use during pregnancy, based on evidence of fetotoxicity and neuroteratogenicity associated with maternal nicotine exposure. Moreover, maternal smoking is associated with numerous adverse cardiovascular, respiratory, endocrine, and metabolic outcomes in the offspring (Beratis *et al*., 1996; Bergmann *et al*., 2003; Blake *et al*., 2000; Jensen *et al*., 1998; Lannero *et al*., 2006; Montgomery and Ekbom, 2002; Oken *et al*., 2008; Power and Jefferis, 2002; Sharpe and Franks, 2002; Syme *et al*., 2009; Toschke *et al*., 2002; Weinberg *et al*., 1989; Wideroe *et al*., 2003), yet the effects of nicotine exposure alone on these outcomes have not been comprehensively evaluated. To date, reviews that evaluate the safety of NRT use during pregnancy generally consider the acute risks of nicotine exposure on the developing fetus and, in some cases, the long-term neurological effects. The goal of this review is to assess the current evidence regarding the long-term effects of fetal and neonatal nicotine exposure, an area of research that has been overlooked in the safety assessment of NRT use during pregnancy. We will also consider the potential contribution of nicotine to the long-term toxicity associated with cigarette smoke exposure during fetal and neonatal development.

NRT USE DURING PREGNANCY: HUMAN TRIALS

There are an extremely limited number of trials examining the safety of NRT in humans, all of which focus on obstetrical outcomes and the short-term toxicological effects on the fetus. A report by Morales-Suarez-Varela *et al*. (2006) showed an increased prevalence of specific malformations in pregnant NRT users compared with both nonsmokers and smokers, whereas Strandberg-Larsen *et al*. (2008) showed in the same cohort (Danish National Birth Cohort) that NRT use during pregnancy was not associated with an increased risk of stillbirth. A recent, open-label randomized trial by Pollak *et al*. (2007), comparing cognitive-behavioral therapy (CBT) with or without NRT, was the first large trial to demonstrate efficacy of NRT for smoking cessation during pregnancy (but not postpartum). Unfortunately, this trial was stopped early because of increased incidence of "serious adverse events" in the CBT + NRT group compared with the CBT arm; however, because of the open-label design of this trial and other confounding factors, it is difficult to make conclusions about these data (Pollak *et al*., 2007). Nicotine gum and patches have been shown to increase maternal blood pressure and heart rate, as well as fetal heart rate, but to a lesser degree than cigarette smoking (reviewed in Dempsey and Benowitz, 2001). Although smoking is clearly associated with intrauterine growth restriction (Kolas *et al*., 2000; Williams *et al*., 1997), the role of nicotine in causing this effect is unclear. Two randomized control trials have found that birth weights were significantly higher in women using nicotine gum (Oncken *et al*., 2008) or nicotine patch (Wisborg *et al*., 2000) compared with smokers receiving placebo controls. Notably, in both of these studies, serum cotinine levels were actually lower in the NRT group than the placebo group (Oncken *et al*., 2008; Wisborg *et al*., 2000). Furthermore, both the NRT and placebo groups included women who quit smoking, those who reduced cigarette consumption, and those who did not change smoking

behaviors, thus making analyses of the role of nicotine alone on birth weight in these studies difficult to interpret (Oncken *et al.*, 2008; Wisborg *et al.*, 2000). Conversely, data from the 2004 Phase V Pregnancy Risk Assessment Monitoring System indicated that self-reported NRT use during pregnancy was associated with a 2-fold increased risk of low birth weight compared with nonsmokers, whereas a 1.3-fold increased risk was observed in smokers versus nonsmokers (Gaither *et al.*, 2009). Therefore, it is difficult to ascertain the role of nicotine in the increased incidence of low birth weight associated with maternal smoking because nicotine exposure in humans may actually be lower in women using NRT. Results from the ongoing smoking, nicotine and pregnancy trial, a double-blind placebo-randomized control trial of NRT in pregnancy (Coleman, 2007), which is expected to end in 2012, should help to clarify the effects of NRT use during pregnancy on birth weight.

Currently, there are no prospective epidemiological studies that examine NRT use during pregnancy and the incidence of adult-onset disease in the offspring. However, considerable insight into the long-term effects of developmental nicotine exposure can be gained from animal models. The general consensus among clinicians is that more information is needed about the risks of NRT use during pregnancy before well-informed definitive recommendations can be made to pregnant women (Crawford *et al.*, 2008; Herbert *et al.*, 2005; Lumley *et al.*, 2009; Oncken and Kranzler, 2009; Osadchy *et al.*, 2009). Although we agree that there is limited information available from human trials with NRT during pregnancy, there is substantial evidence in animal models that can contribute to dialogue on this subject. Therefore, this review will evaluate a broader spectrum of outcomes than has been previously examined, including the long-term consequences of fetal and neonatal nicotine exposure in animal studies and the potential contribution of nicotine to the increased incidence of adult-onset diseases in humans following maternal smoking during pregnancy.

LONG-TERM EFFECTS OF FETAL AND NEONATAL EXPOSURE TO NICOTINE

Neurobehavioral Outcomes

The long-term consequences of fetal and neonatal exposure to nicotine on the central nervous system have been extensively studied in numerous animal models and are reviewed in detail elsewhere (reviewed in Dwyer *et al.*, 2009; Pauly and Slotkin, 2008; Winzer-Serhan, 2008). Briefly, nicotine has been clearly established as a neuroteratogen that compromises the development of critical neural pathways in the developing brain (Dwyer *et al.*, 2009; Pauly and Slotkin, 2008; Winzer-Serhan, 2008). Numerous long-term neurological effects have also been documented following prenatal nicotine exposure, which are thought to explain many of the adverse neurobehavioral outcomes in the offspring of women who smoke during pregnancy. For instance, epidemiological studies have demonstrated that prenatal tobacco exposure is associated with numerous adverse postnatal neurobehavioral outcomes, such as attention-deficit hyperactivity disorder, learning disabilities, behavioral problems, and increased risk of nicotine addiction (reviewed in Cornelius and Day, 2009; Dwyer *et al.*, 2009; Pauly and Slotkin, 2008; Rogers, 2009; Winzer-Serhan, 2008). Similarly, prenatal nicotine exposure in rodents causes postnatal hyperactivity, cognitive impairment, increased anxiety, somatosensory deficits, persistent neurochemical alterations, changes in sensitivity to nicotine, alterations in nicotine self-administration and altered patterns of neural cell survival, and synaptogenesis (reviewed in Dwyer *et al.*, 2009; Pauly and Slotkin, 2008; Winzer-Serhan, 2008). Evidence from animal studies strongly points to nicotine as a key chemical involved in mediating the long-term neurological effects of developmental cigarette smoke exposure.

Metabolic Outcomes

Because it is well established that maternal cigarette smoking results in intrauterine growth restriction (Andres and Day, 2000; England *et al.*, 2001; Robinson *et al.*, 2000; Wideroe *et al.*, 2003) and that low birth weight is a significant risk factor for the development of obesity, hypertension, and type 2 diabetes (Barker, 1998; Barker and Clark, 1997; Godfrey and Barker, 2000, 2001; Ong and Dunger, 2002; Seckl, 2001), it has been suggested that the association of cigarette smoking with an increased risk of adverse postnatal health outcomes is simply a reflection of intrauterine growth restriction. However, maternal smoking increases the risk of adult-onset diseases in the offspring even after adjustment for a wide range of confounding factors including birth weight, socioeconomic status, and maternal diet (Power and Jefferis, 2002; Syme *et al.*, 2009; Von *et al.*, 2002; Wideroe *et al.*, 2003), suggesting that it may be a direct effect of intrauterine exposure to the chemicals in cigarette smoke that accounts for the increased risk of adverse health outcomes in the offspring of women who smoke during pregnancy. Indeed, recent epidemiological studies have shown a strong relationship between maternal smoking and subsequent obesity, hypertension, and type 2 diabetes in the offspring (Bergmann *et al.*, 2003; Montgomery and Ekbom, 2002; Oken *et al.*, 2008; Power and Jefferis, 2002; Syme *et al.*, 2009; Toschke *et al.*, 2002; Wideroe *et al.*, 2003). Of the 4000 chemicals in cigarette smoke, animal studies suggest that fetal exposure to nicotine alone may result in postnatal metabolic alterations associated with obesity, type 2 diabetes, and hypertension. This hypothesis is supported by work from our laboratory, which demonstrated that maternal nicotine exposure during pregnancy and lactation in rats results in increased adiposity and/or increased body weight (Gao *et al.*, 2005; Holloway *et al.*, 2005), altered perivascular adipose tissue composition and function (Gao *et al.*, 2008), elevated blood pressure (Gao *et al.*,

2008), and impaired glucose homeostasis (Holloway *et al.*, 2005) postnatally. In our animal model, female rats are injected daily with either nicotine bitartrate (1.0 mg/kg/day) or saline (vehicle) for 2 weeks prior to mating, 3 weeks during gestation (fetal development), and 3 weeks during lactation (neonatal development). The dose of nicotine used in our animal model results in maternal serum cotinine concentrations of 136 ng/ml (Holloway *et al.*, 2006), which is within the range of cotinine levels reported in women who are considered ''moderate smokers'' (80–163 ng/ml) (Eskenazi and Bergmann, 1995). In addition, this dose of nicotine results in serum cotinine concentrations of 26 ng/ml in the nicotine-exposed offspring at birth (Holloway *et al.*, 2006), which is also within the range (5–30 ng/ml) observed in infants nursed by smoking mothers (Luck and Nau, 1985). The following sections will evaluate findings from our animal model as well as similar models of developmental nicotine exposure by others in the field.

Obesity. Prenatal nicotine exposure in rats has been shown to result in increased postnatal body weight (Gao *et al.*, 2005; Newman *et al.*, 1999; Somm *et al.*, 2008) and higher levels of body fat in the fetus at gestational day 20 (Williams and Kanagasabai, 1984) and offspring during adulthood (Gao *et al.*, 2005; Oliveira *et al.*, 2009; Somm *et al.*, 2008). It has been suggested that prenatal nicotine exposure may result in increased adiposity/body weight via alterations in the central endocrine control of body weight homeostasis. The signals for body weight regulation and energy balance are ultimately integrated in the hypothalamus, which is arguably the most important center in the brain for the regulation of appetite and body weight homeostasis (Hillebrand *et al.*, 2002; Kalra *et al.*, 1999; Wilding, 2002). Wideroe *et al.* (2003) suggested that the underlying mechanism for the reported association between maternal smoking and childhood overweight may be inappropriate changes in the hypothalamic regulation of energy homeostasis resulting in increased appetite. Indeed, nicotinic acetylcholine receptors (nAChR) are widely distributed in the hypothalamus (Jo *et al.*, 2002; Li *et al.*, 2003), and Grove *et al.* (2001) have shown that *in utero* exposure to nicotine in the rhesus macaque can alter hypothalamic expression of both neuropeptide Y and proopiomelanocortin (POMC) messenger RNA (mRNA), regulators of appetite and satiety, in the neonate. Similarly, neonatal rats treated with nicotine also have significantly upregulated levels of POMC mRNA in the arcuate nucleus, an effect that was blocked by dihydro-β-erythroidine, an α4β2 nAChR antagonist (Huang and Winzer-Serhan, 2007). These results suggest that the nicotine-induced alterations in body weight homeostasis may be regulated by changes in hypothalamic control mechanisms during fetal life.

Type 2 diabetes. We have demonstrated that in rats, nicotine exposure alone, during pregnancy and lactation, results in endocrine and metabolic changes in the adult offspring (i.e., 26 weeks of age) that are consistent with the disturbed glucose metabolism that may lead to type 2 diabetes (Holloway *et al.*, 2005). In humans, type 2 diabetes results from a progressive reduction in the ability of the pancreas to produce sufficient insulin to maintain normal glucose homeostasis and compensate for any underlying resistance to the action of insulin (Butler *et al.*, 2003; Marchetti *et al.*, 2006). Impaired insulin secretion may be because of a reduced number of insulin-secreting cells (i.e., beta cell mass) and/or abnormal insulin secretion from the beta cells (i.e., beta cell function). Insulin resistance, a reduced sensitivity to insulin action, is also a major component of the pathophysiology of type 2 diabetes and can be characterized by defective insulin signaling in insulin target tissues and/or impaired insulin-stimulated glucose transport in skeletal muscle.

In rats, fetal and neonatal exposure to nicotine adversely affects pancreatic development and postnatal beta cell survival and function (Bruin *et al.*, 2007b, 2008a,b,c). Beta cell mass is determined by a balance of beta cell size, replication, neogenesis, and apoptosis (Bouwens and Rooman, 2005; Hill, 2005; Rhodes, 2005). We have demonstrated that fetal and neonatal nicotine exposure caused a permanent loss of beta cell mass beginning at birth, which was attributed to increased levels of beta cell apoptosis and a decreased capacity for islet cell proliferation compared with saline controls (Bruin *et al.*, 2007b). This is similar to humans with type 2 diabetes, in which the primary cause of impaired beta cell mass is increased apoptosis (Butler *et al.*, 2003). Furthermore, Somm *et al.* (2008) have assessed the effects of prenatal nicotine exposure on beta cell neogenesis in a similar animal model. In their study, fetal and neonatal exposure to nicotine resulted in reduced islet size and number, as well as a reduction in beta cell neogenesis, as determined by impaired islet gene expression of Pdx-1, Pax-6, Nkx6.1, and insulin (Somm *et al.*, 2008). Regardless of the treatment protocol, nicotine-exposed offspring subsequently developed glucose and insulin intolerance, hyperinsulinemia, and increased body weight during adulthood (Holloway *et al.*, 2005; Somm *et al.*, 2008). Taken together, these studies show clearly that in animals, fetal and neonatal exposure to nicotine has profound effects on fetal and neonatal pancreatic development resulting in abnormal glucose homeostasis in adulthood.

Our laboratory has further examined the mechanism by which fetal and neonatal nicotine causes loss of beta cell mass and function. We propose that nicotine binds to the nAChR on the developing beta cell, causing an increase in the production of intracellular reactive oxygen species (Bruin *et al.*, 2008b). Because the antioxidant defense system in the beta cells is known to be relatively low compared with other cell types (Lenzen *et al.*, 1996; Tiedge *et al.*, 1997), beta cells are particularly susceptible to oxidative stress. Notably, we have shown increased oxidative damage specifically to mitochondrial proteins in the pancreas of nicotine-exposed neonates (Bruin *et al.*, 2008b). Indeed, mitochondrial dysfunction appears to be a central defect in beta cells following nicotine exposure. First, loss of beta cell mass in nicotine-exposed

neonates was attributed to increased mitochondrial-mediated beta cell apoptosis (Bruin *et al.*, 2008a). Second, a progressive deterioration of mitochondrial structure and function was observed with increasing age following cessation of nicotine treatment at weaning (Bruin *et al.*, 2008c). Finally, these mitochondrial defects were associated with impaired glucose-stimulated insulin secretion in isolated islets and reduced insulin granule biosynthesis (Bruin *et al.*, 2008c), both of which likely contributed to the altered glucose homeostasis in this animal model.

Prenatal nicotine exposure also causes impaired insulin sensitivity in the peripheral tissues, another hallmark of type 2 diabetes. Somm *et al.* (2008) reported an increased glucose response following an insulin challenge in nicotine-exposed offspring relative to saline controls during adulthood. Furthermore, we have shown significantly reduced insulin receptor protein expression (Bruin *et al.*, 2007a) and uptake of radiolabeled insulin (Labiris *et al.*, 2006) in skeletal muscle at 26 weeks of age following fetal and neonatal nicotine exposure. Therefore, maternal nicotine exposure may cause impaired glucose homeostasis in offspring as a result of both defective insulin secretion (caused by impaired pancreatic beta cell mass and function) and reduced peripheral insulin sensitivity.

Cardiovascular Outcomes

Hypertension is one of the health consequences associated with *in utero* exposure to cigarette smoking in humans (Beratis *et al.*, 1996; Blake *et al.*, 2000), and animal studies suggest that this effect may be mediated via nicotine. Indeed, fetal and neonatal nicotine exposure results in increased blood pressure during adulthood in both the normotensive Wistar-Kyoto rat strain (Gao *et al.*, 2008) and a spontaneously hypertensive strain (Pausova *et al.*, 2003). However, the mechanisms for blood pressure elevation in the offspring of the nicotine-exposed dams are not fully understood.

Results from other studies suggest that fetal and neonatal exposure to nicotine in rats causes elevated blood pressure postnatally because of endothelial dysfunction (Xiao *et al.*, 2007) and/or changes in renal structure or function (Mao *et al.*, 2009; Pausova *et al.*, 2003). However, in our animal model, there was no evidence of either endothelial dysfunction or altered renal structure in the nicotine-exposed offspring at 6 months of age (Gao *et al.*, 2008). Instead, data from these experiments have shown that fetal and neonatal exposure to nicotine (1) changes the composition and amount of perivascular adipose tissue and (2) impairs the ability of the perivascular adipose tissue to attenuate the contractile response of blood vessels (Gao *et al.*, 2005, 2008).

Because perivascular adipose tissue surrounds almost all systemic arteries and is an important modulator of vascular function (Gao *et al.*, 2007; Gollasch and Dubrovska, 2004), these data suggest that the elevated postnatal blood pressure in nicotine-exposed offspring may be at least partly attributed to altered perivascular adipose tissue function.

Prenatal nicotine exposure also leads to stress-induced cardiac defects in the offspring. Nicotine-exposed rat offspring had elevated left ventricle myocardial infarct size and decreased postischemic recovery of left ventricle function following 25 min of ischemia during adulthood (Lawrence *et al.*, 2008). Similarly, intolerance to neonatal hypoxia has been attributed to impaired maintenance of cardiac function following prenatal nicotine exposure (Slotkin *et al.*, 1997). Control rats responded to hypoxic conditions with initial tachycardia and a subsequent slight decline in heart rate, whereas nicotine-exposed pups show no tachycardia and a rapid decline in heart rate (Slotkin *et al.*, 1997). These effects also extended into adulthood with nicotine-exposed rats showing a higher incidence of arrhythmia in response to stress at 4–5 months of age compared with saline controls (Feng *et al.*, 2010). Taken together, animal studies demonstrate that developmental nicotine exposure may play a key role in the increased risk of hypertension following maternal smoking (Beratis *et al.*, 1996; Blake *et al.*, 2000). Furthermore, nicotine-induced cardiac dysfunction may also be involved in the increased risk of SIDS in cigarette smoke–exposed neonates (Mitchell and Milerad, 2006).

Respiratory Outcomes

Lung development. Epidemiological studies have associated maternal cigarette smoke exposure with a variety of adverse pulmonary function outcomes in the offspring. Maternal smoking during pregnancy doubles the risk of wheezing and asthma in offspring up to 2 years of age (Lannero *et al.*, 2006) and is associated with diminished lung function parameters in children (Gilliland *et al.*, 2000). The risk of the offspring developing asthma following maternal smoking during pregnancy remains elevated as the children reach school age, adolescence, and adulthood (Gilliland *et al.*, 2001; Skorge *et al.*, 2005). In addition, maternal smoking during pregnancy synergizes with personal smoking in later life to increase airflow limitations in chronic obstructive pulmonary disease (Upton, 2004).

Similarly, perinatal exposure to nicotine also has a profound impact on lung development and postnatal lung function. Because this topic has been reviewed in detail elsewhere (Campos *et al.*, 2009; Hafstrom *et al.*, 2005; Maritz, 2008), we will only highlight selected studies. For instance, nicotine can alter airway structure and mechanics in fetal monkeys, resulting in decreased pulmonary function parameters such as forced expiratory volume, forced vital capacity, and expiratory reserve volume (Sekhon *et al.*, 1999, 2001, 2002). In addition, nicotine impairs alveolarization in the lungs of perinatally exposed rats (Maritz, 2002; Maritz and Thomas, 1994; Maritz and Windvogel, 2003; Petre *et al.*, 2008). Offspring of nicotine-treated lambs also had abnormal postnatal breathing patterns (Hafstrom *et al.*, 2002a) and proximal airway obstruction (Sandberg *et al.*, 2004). However, the long-term

consequences of these changes in lung structure have not been well described. Maritz and Windvogel (2003) have followed offspring from their studies up to postnatal day 42 and have observed accelerated aging of the lungs, characterized by microscopic emphysema, enlarged alveolar volume, increased flattening of alveoli with age, and decreased internal surface area for gas exchange. Similarly, in our laboratory, nicotine exposure resulted in altered alveolarization and reduced lung vascularization in the neonates, suggesting that gas exchange might be compromised. However, these changes in lung structure in neonatal life did not translate into permanent functional changes in lung mechanics or airway responsiveness to a methacholine challenge during adulthood (Petre *et al*., 2008).

Hypoxia sensing. Cigarette smoking during pregnancy is associated with an increased risk of SIDS (Mitchell and Milerad, 2006). Indeed, exposure to nicotine alone during gestation in rats results in increased mortality during a hypoxic challenge on the day after birth (Slotkin *et al*., 1995). As previously discussed, this effect has been attributed, in part, to the adverse consequences of maternal nicotine on the maintenance of postnatal cardiac function under conditions of ischemic stress (Lawrence *et al*., 2008; Slotkin *et al*., 1997). In addition, studies in lambs have shown that prenatal nicotine exposure alters the lung mechanical response to hypoxia (Sandberg *et al*., 2007) and blunts the major cardiorespiratory defense systems to hypoxia (i.e., heart rate and ventilatory and arousal responses) (Hafstrom *et al*., 2002b). It has been proposed that these defects may all be attributed to the deficient adrenomedullary catecholamine release observed during hypoxia in nicotine-exposed neonatal rats (Slotkin *et al*., 1995). Indeed, in our animal model, there is an impaired ability of adrenomedullary chromaffin cells to respond to hypoxic stress following fetal nicotine exposure (Buttigieg *et al*., 2008). Nicotine treatment of primary chromaffin cells (isolated from neonates) in culture blunted hypoxia-induced catecholamine secretion (Buttigieg *et al*., 2009). Similarly, significantly lower levels of catecholamines were detected in umbilical cord blood of smokers compared with nonsmokers (Oncken *et al*., 2003). Therefore, prenatal nicotine exposure blunts the neonatal response to hypoxia via a combination of respiratory, cardiovascular, and adrenal chromaffin cell defects.

Fertility Outcomes

It has been well documented that there is a significant association between smoking and reduced fertility among females (Augood *et al*., 1998; Greenlee *et al*., 2003; Hughes and Brennan, 1996; Hull *et al*., 2000; Shiverick and Salafia, 1999) and males (Kunzle *et al*., 2003; Vine, 1996). Furthermore, cotinine (the metabolite of nicotine), cadmium (a heavy metal in cigarette smoke), and benzo[*a*]pyrene (a polyaromatic hydrocarbon in cigarette smoke) have been detected in the follicular fluid of women who smoke (Neal *et al*., 2008; Younglai *et al*., 2002; Zenzes *et al*., 1995), demonstrating that chemicals present in cigarette smoke can accumulate in the ovary. It has been suggested that impaired fertility in women who smoke may be the result of a combination of impaired oocyte function and viability, decreased fertilization rates, altered ovarian steroidogenesis, depleted ovarian reserves, and increased chromosomal abnormalities in oocytes (Harrison *et al*., 1990; Klonoff-Cohen *et al*., 2001; Ness *et al*., 1999; Van Voorhis *et al*., 1996; Zenzes *et al*., 1995).

In human populations, there is also evidence to suggest that fetal exposure to cigarette smoke is associated with reduced fertility during adulthood in both men and women (Jensen *et al*., 1998; Sharpe and Franks, 2002; Weinberg *et al*., 1989). Data from animal studies suggest that nicotine exposure may be a critical component in the development of adverse reproductive effects in the offspring of women who smoke. In our animal model, nicotine exposure during fetal and neonatal development resulted in reduced fertility, dysregulation of ovarian steroidogenesis, and altered follicle dynamics in female offspring (Holloway *et al*., 2006). Furthermore, developmental nicotine exposure resulted in reduced granulosa cell proliferation, increased ovarian cell apoptosis, and decreased ovarian angiogenesis during adulthood compared with saline controls (Petrik *et al*., 2009), an effect that may be mediated, in part, via changes in the intra-ovarian growth insulin-like growth factor system (Cesta *et al*., 2009).

The results for male offspring appear to be less profound than the changes observed in the females. There were some transient defects noted in the histopathology of nicotine-exposed testes during the peripubertal period (7 weeks of age), including increased spermatid retention, seminiferous tubule vacuolization, leukocyte and germ cell infiltration into epididymal ducts, and germ cell exfoliation and depletion in seminiferous tubules (Anzar *et al*., 2006). However, these structural changes were not evident during adulthood (i.e., 26 weeks of age) and were not associated with adverse functional outcomes at either age (Lagunov *et al*., 2009). Therefore, fetal and neonatal nicotine exposure appears to play an important role in the infertility reported in female offspring of smoking mothers, but the role in male offspring remains unclear. This is consistent with a study in Danish dizygotic twins, which reported that the female twin had reduced fecundity following *in utero* cigarette smoke exposure, whereas the fecundity of the male twin was unaffected (Jensen *et al*., 2006).

Childhood Cancers

Prenatal exposure to tobacco smoke has been associated with an increased risk of childhood cancers, including childhood brain tumors and leukemia/lymphoma (Sasco and Vainio, 1999). The long-term effects of fetal and neonatal nicotine exposure on cancer development are not well studied, but there is certainly biological plausibility to suggest that this may be an area of risk. Nicotine and its metabolites are known to both initiate and promote tumor growth (Catassi *et al*., 2008; Martin *et al*., 2009; Zheng *et al*., 2007). The fetus may be particularly

vulnerable to these effects because of its reduced detoxification abilities (Perera *et al*., 2004).

Nicotine can be transformed to the carcinogenic compound, the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Notably, although NRT users have reduced urinary levels of NNK metabolites compared with smokers, nicotine from NRT was transformed to these tobacco-specific carcinogens (Hatsukami *et al*., 2004). NNK and its metabolites have been detected in the urine of newborns born to mothers who smoked cigarettes during pregnancy, indicating placental transfer of this carcinogen (Lackmann *et al*., 1999). It can be assumed that women using NRT during pregnancy would also expose the fetus to NNK, although likely at lower levels. Notably, NNK is a transplacental carcinogen in the Syrian golden hamster, even at low doses (Schuller *et al*., 1994). Offspring of pregnant hamsters treated with NNK develop tumors in various different tissues, including the respiratory tract, pancreas, liver, and adrenal glands (Correa *et al*., 1990; Schuller *et al*., 1993, 1994). Therefore, fetal and neonatal exposure to nicotine, via NRT or cigarette smoking, may similarly increase the long-term risk of developing cancer, although more research is needed to verify this hypothesis.

CONCLUSIONS

Although the human literature about the long-term effects of NRT use during pregnancy is lacking, there is substantial evidence from animal studies indicating that fetal and neonatal nicotine exposure leads to widespread adverse postnatal health consequences. For instance, in our animal model alone, maternal nicotine exposure during gestation and lactation caused defective metabolic (Bruin *et al*., 2007b, 2008a,b,c; Holloway *et al*., 2005), reproductive (Anzar *et al*., 2006; Holloway *et al*., 2006; Lagunov *et al*., 2009), cardiovascular (Gao *et al*., 2005, 2008), pulmonary (Petre *et al*., 2008), and hypoxia-sensing (Buttigieg *et al*., 2008, 2009) outcomes in the offspring. Furthermore, the adverse effects of maternal nicotine are not limited to the first generation (F1) offspring but also appear to affect the second generation (F2) (Holloway *et al*., 2007). F2 offspring whose mothers were exposed to nicotine during development had elevated blood pressure, increased fasting serum insulin, and an enhanced insulin response to an oral glucose challenge (Holloway *et al*., 2007). Taken together with the vast literature on the long-term effects of developmental nicotine exposure in other animal models, these results have significant clinical implications and should be considered by health associations when making policy decisions about the safety of NRT use during pregnancy. Overall, the evidence provided in this review overwhelmingly indicates that nicotine should no longer be considered the "safe" component of cigarette smoke. In fact, many of the adverse postnatal health outcomes associated with maternal smoking during pregnancy may be attributable, at least in part, to nicotine alone.

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