

# Maternal Smoking During Pregnancy & Animal Studies of Developmental Exposure to Nicotine

(version updated January 3, 2011)

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## Maternal Smoking During Pregnancy

Smoking during pregnancy is a known risk factor for low birth weight or small for gestational age (Lumley *et al.* 2008; Oken *et al.* 2008), including across different categories of maternal BMI and when the pregnancy was complicated by diabetes and hypertension (Aagaard-Tillery *et al.* 2008).<sup>1</sup> Approximately 20 epidemiological studies have looked at the impact of maternal smoking during pregnancy and body weight in offspring during childhood or adulthood. These studies show a consistent association between maternal smoking during pregnancy and increased risk of overweight/obesity in the offspring (Figure 1 and Appendix Table A). This literature was evaluated in two recent meta-analyses (Ino 2010; Oken *et al.* 2008).<sup>2</sup> The pooled OR estimate in Oken *et al.* (2008) for elevated risk of overweight was 1.50 (95% CI: 1.35-1.65) based on 14 studies. The pooled OR in Ino (2010) for obesity (BMI > 95<sup>th</sup> percentile) was 1.64 (1.42-1.90) based on 16 studies.

Women who smoked during pregnancy tended to have low social/economic status, less education, weight more, and were less likely to breastfeed (Ino 2010; Oken *et al.* 2008). Their

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<sup>1</sup> Maternal smoking was not found to be associated with nonanomalous still births based on data from 1566 nonanomalous still births and 2720 nonanomalous controls identified from Utah Birth and Fetal Death Certificates from 1992-2002 (Aagaard-Tillery *et al.* 2006)

<sup>2</sup> Oken *et al.* (2008) included 14 studies in the meta-analysis: (Adams *et al.* 2005; Al Mamun *et al.* 2006; Bergmann *et al.* 2003; Chen *et al.* 2006; Dubois and Girard 2006; Oken *et al.* 2005; Power and Jefferis 2002; Reilly *et al.* 2005; Salsberry and Reagan 2005; Toschke *et al.* 2002; Toschke *et al.* 2003; von Kries *et al.* 2002; Whitaker 2004; Widerøe *et al.* 2003)

Ino (2010) included 17 studies in the meta-analysis: (Adams *et al.* 2005; Al Mamun *et al.* 2006; Bergmann *et al.* 2003; Chen *et al.* 2006; Dubois and Girard 2006; Huang *et al.* 2007; Mizutani *et al.* 2007; Oken *et al.* 2005; Power and Jefferis 2002; Reilly *et al.* 2005; Salsberry and Reagan 2005; Tome *et al.* 2007; Toschke *et al.* 2002; Toschke *et al.* 2003; von Kries *et al.* 2002; Whitaker 2004; Widerøe *et al.* 2003)

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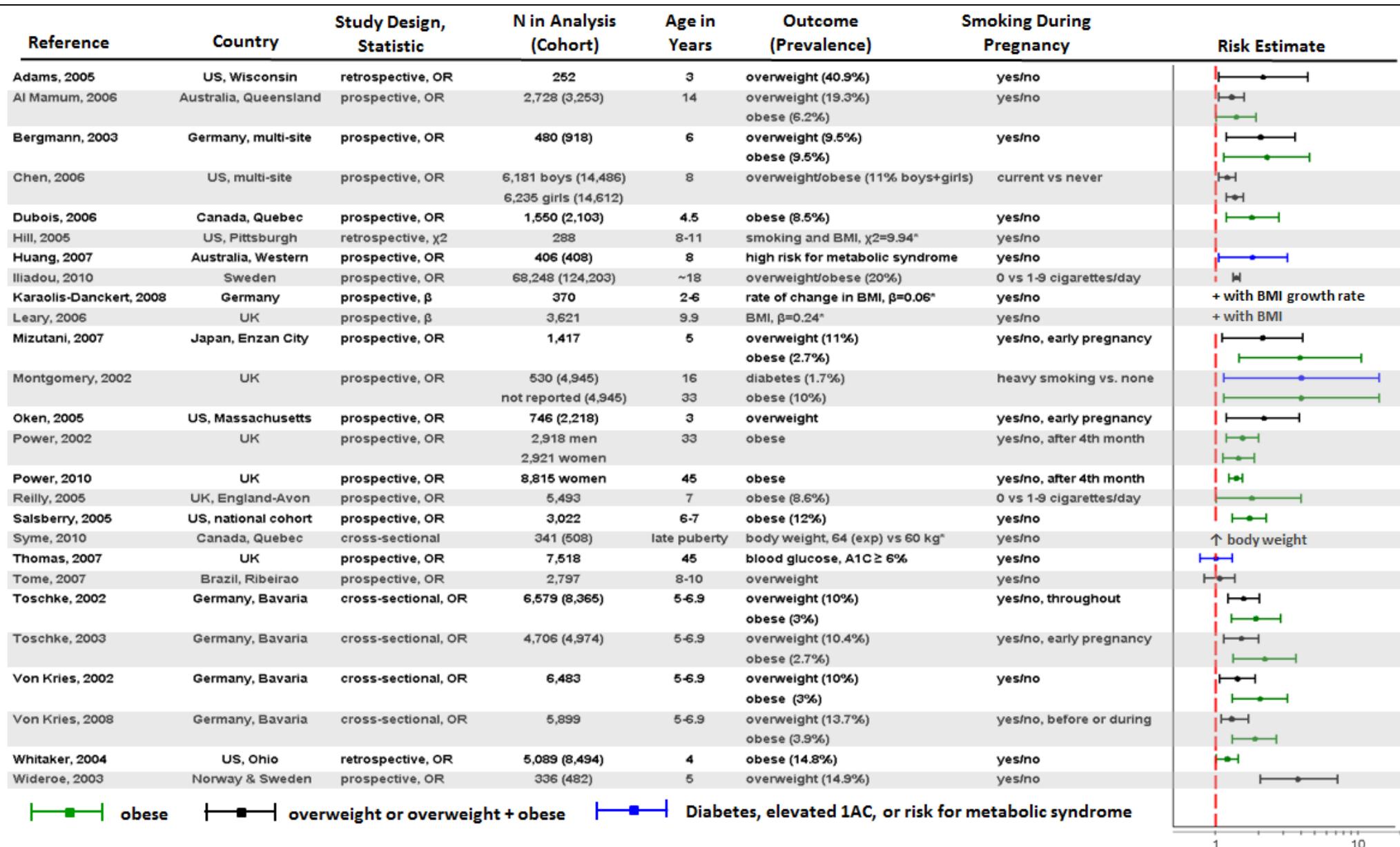
children had more rapid weight gain during infancy and were less active (Oken *et al.* 2008). These differences between smokers and non-smokers were less apparent in older or European cohorts where smoking was more common. But inclusion of these variables into the regression models did not greatly impact the ORs and the associations with overweight were independent of birth weight, fetal growth, or postnatal weight gain (Oken *et al.* 2008). Crude and adjusted ORs were similar for studies [(Oken *et al.* 2008), see also [Appendix Table A](#)]. This suggests that social and behavioral differences between smokers and nonsmokers are not likely to account for the observed differences in overweight risk. The timing of smoking exposure was important. In general, smoking throughout pregnancy was associated with a greater risk for child overweight than was smoking in early pregnancy only.

Both meta-analyses used funnel plot methods to ascertain publication bias and concluded there was some evidence for publication bias, but not enough to negate the overall conclusion of increased risk. Adjusted pooled ORs that considered publication bias were still significant: OR (95% CI) = 1.40 (1.26-1.55) (Oken *et al.* 2008) and 1.52 (1.36-1.70) (Ino 2010). Oken *et al.* (2008) also repeated the analysis on a more limited set of findings that excluded studies where the mother was asked about prenatal smoking habits at the time the outcomes were assessed in offspring (rather than during pregnancy or at birth). The pooled estimate did not appreciably change (OR 1.51, 95% CI: 1.35, 1.70), suggesting minimal impact of recall bias.

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Figure 1. Summary of studies assessing maternal smoking and overweight or obesity in offspring



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One of the studies presented in [Figure 1](#), Tome et al. (2007), did not report a significant association between maternal smoking and risk of offspring overweight at 8 to 10 years of age (adjOR = 1.07; 95%CI =0.84-1.37). This study was conducted in the Ribeirao region of Brazil and 9.5% of the children were malnourished (BMI < 5<sup>th</sup> percentile). While the association with maternal smoking was not significant for overweight, the authors found smoking during pregnancy to be “protective” against malnutrition. From this perspective, the study findings are consistent with the broader literature.

Although the crude and adjusted ORs are similar for most studies, results from some of the studies demonstrate the challenges of fully disentangling the effect of maternal smoking from confounding variables and other factors. Iliadou et al. (2010) looked at the relationship between maternal smoking and BMI in 124,203 males born between 1983-1988 identified in the Swedish Medical Birth Register. BMI data was obtained via military conscription records and maternal smoking data is included in the Medical Birth Register. They found the predicted association of increased risk for overweight (BMI ≥25) across the entire cohort: adjusted OR (95%CI) of 1.41 (1.34–1.49), and 1.56 (1.46–1.66), for 1-9 cigarettes per day and > 10 cigarettes per day, respectively. But, they also conducted stratified analyses using sibling pairs that led them to conclude that the association was partly confounded by unmeasured familial factors.<sup>3</sup> Specifically, the increased risk of overweight in the 2<sup>nd</sup> son was only observed if the mother smoked during both pregnancies ([Table 1](#)). Also, the authors calculated “within-family” scores for half and full siblings to capture the difference between smoking status at the first pregnancy and mean value of smoking status across the 2 pregnancies. This score compares exposure to maternal smoking during pregnancy relative to the sibling’s exposure. Within-family associations were evaluated for both full and half-siblings to determine whether or not common genes are likely to confound the association (on average, full and half siblings share 50 and 25% of segregating genes). The argument is that the association is likely to be confounded by common genetic factors if the size of the within effect of maternal smoking during pregnancy and offspring BMI is weaker in half compared with full siblings. The adjusted<sup>4</sup> regression coefficients for the within-family component, expressed as expected change in offspring BMI for a one unit change in maternal smoking behavior between pregnancies, were smaller for half-siblings [0.07 (95% CI –4.32 to 4.45); n=138] compared to full-siblings [0.39 (95% CI –0.01 to 0.78); n=9,243], but the difference was not statistically significant (p=0.79). The within family associations of 0.07 and 0.39 were not statistically significant which indicated that the effect of smoking on offspring’s BMI was not present in the full and half-sibling pairs.

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<sup>3</sup> Analysis based on the overall cohort as well as stratifying for maternal smoking habits during first trimester across 2 subsequent male pregnancies.

<sup>4</sup> Adjusted for maternal age, height, BMI, pregnancy weight gain, maternal and paternal socio-economic category and education and offspring birth weight, head circumference, gestational age, urban living and age at conscription

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**Table 1. Maternal smoking in first and second born son and the risk of overweight in siblings based on 8441 women with subsequent male births between 1983 and 1988**

Maternal smoking during pregnancy		First son			Second son		
1 <sup>st</sup>	2 <sup>nd</sup>	Total n	Overweight n	adjOR (95% CI)	Total n	Overweight n	adjOR (95% CI)
no	no	6190	979	1.00	6190	1078	1.00
yes	no	524	96	1.19 (0.87-1.63)	524	113	1.20 (0.88-1.65)
no	yes	228	46	1.15 (0.75-1.78)	228	51	0.96 (0.58-1.57)
yes	yes	1499	366	1.65 (1.35-2.01)	1499	400	1.71 (1.39-2.09)

Reprinted from Iliadou et al. (2010) with permission from publisher.

von Kries et al. (2008) found that paternal smoking was almost equally as important a risk factor as maternal smoking. The unadjusted OR for obesity (BMI  $\geq$  97<sup>th</sup> percentile) and paternal smoking was 2.0 (95% CI: 1.5-2.6) and 1.3 (95% CI: 0.9-1.9) after adjustment, including for maternal smoking status at an interview (conducted when the children were entering school). The unadjusted OR for obesity and maternal smoking was 2.3 (95% CI: 1.8,-3.1). The unadjusted ORs between obesity and paternal and maternal smoking were of similar magnitude, although consideration of paternal smoking status at the time of the interview and other confounding factors could only partially explain the effect of maternal smoking [adjusted OR1.9 (95% CI: 1.3, 2.7) for before or in pregnancy on childhood obesity]. Exposure to second hand smoke was not characterized. Leary et al. also found significant associations between partner smoking status during pregnancy and offspring BMI and total fat mass at 9.9 years of age. The associations were only weaker than the associations with maternal smoking but still statistically significant.<sup>5</sup> Thus it may be that the association with maternal smoking reflects residual confounding, at least partly, or a direct association with tobacco, either from maternal smoking or exposure to second hand smoke from paternal smoking.

It should be noted that other early-life factors have also been associated with later overweight and obesity: maternal diabetes, paternal smoking, rapid infant, growth, no or short breastfeeding, obesity in infancy, short sleep duration, <30 min of daily physical activity, and consumption of sugar-sweetened beverages (Monasta *et al.* 2010). Other studies suggest that variations in growth in the offspring of women who smoked during pregnancy may result from individual differences in the ability of the fetus to excrete reactive metabolites of compounds found in tobacco. This may stem from polymorphisms that result in enzymatic inactivity of the phase II metabolic enzyme, such as glutathione S-transferase (GSTT1) (Aagaard-Tillery *et al.* 2010). Maternal smoking also may alter placental CYP1A1 expression via epigenetic mechanisms (Suter *et al.* 2010); Suter, 2010 #2023}.

<sup>5</sup> Regressions of offspring BMI and total fat at mean age 9.9 years and maternal and partner smoking status (n=3,606)

	adjusted $\beta$ (95%CI)	
	BMI	total fat
maternal smoking	0.24 (0.16-0.32), p<0.001	0.19 (0.12-0.26), p<0.001
partner smoking	0.11 (0.05-0.18), p=0.001	0.08 (0.02-0.14), p=0.01

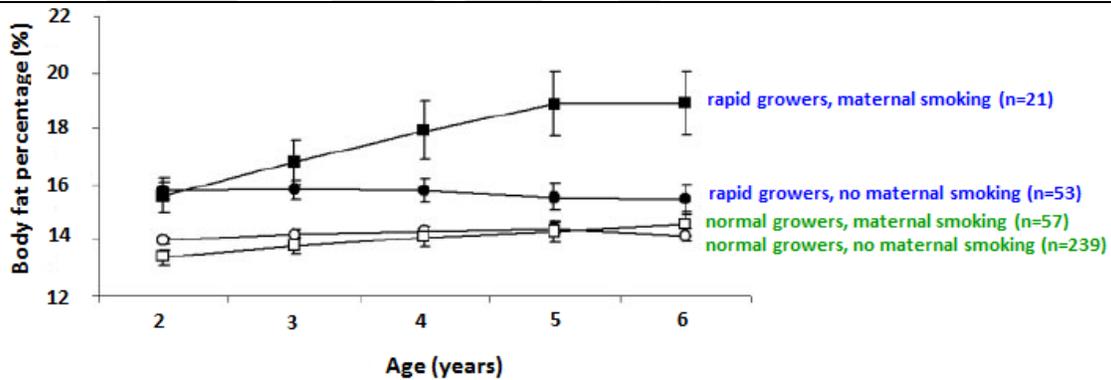
Adjusted for sex and child's age at DXA scan; maternal, partner, social, and infant feeding factors; birth weight and gestation  
From Leary et al. (2006)

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Maternal smoking status is an important factor to consider in the studies that look at associations between exposures to environmental chemicals during development and later onset of overweight or obesity, both as a confounding variable and effect modifier. For example, Verhulst et al (2009) reported a small association between DDE on BMI at 3 years of age in mother’s who didn’t smoke during pregnancy (difference in BMI standard deviation scores for DDE concentrations between the 90th and 10th percentiles = 0.13), but an enhanced relationship when maternal smoking was present (difference in BMI standard deviation scores for DDE concentrations between the 90th and 10th percentiles = 0.13).

Less data is available on more focused measures of adiposity in offspring after infancy (e.g., percent body fat, skin fold thickness) or levels of [leptin](#) or [adiponectin](#), two key hormones involved in regulating appetite and energy expenditure (Table 2). Leptin levels measured in cord blood or blood collected from very young infants are no different or lower in maternal smoking groups compared to non-smoking groups (Coutant *et al.* 2001; Helland *et al.* 2001; Kayemba-Kay’s *et al.* 2008; Mantzoros *et al.* 1997; Ozkan *et al.* 2005; Pardo *et al.* 2004; Vatten *et al.* 2002). One study measured cord blood for adiponectin and reported a significant negative correlation with number of cigarettes smoked per day,  $r = -0.245$ ;  $p = 0.01$  (Pardo *et al.* 2005). The mean levels of adiponectin did not differ in full-term infants across smoking status groups but were significantly lower in premature infants of women who smoked during pregnancy (16.34  $\mu\text{g/ml}$ ;  $n=7$ ) versus 23.28  $\mu\text{g/ml}$  in infants of mothers who did not smoke ( $n=10$ ). Karaolis-Danckert et al. (2008) looked at the interaction of exposure to tobacco *in utero* and percent body fat in children between the ages of 2 and 6 years. They found a significant interaction based on the degree of weight change prior to 2 years, such that maternal smoking only seemed to impact percent body in children who displaying “rapid” weight gain during the first 2 years of life (Figure 2).<sup>6</sup>

**Figure 2. Rapid growers exposed to tobacco *in utero* subsequently gained more percent body fat between 2 and 6 years of age than did rapid growers who had not been exposed**



Predicted mean ( $\pm$ SEM) percentage body fat trajectories by subgroups of rapid weight gain and intrauterine tobacco exposure and maternal overweight status in 370 children in the German Multicenter Allergy Study. The plot shows the 3-factor interaction between rapid weight gain and intrauterine tobacco exposure ( $\beta \pm$  SE:  $0.78 \pm 0.28\%/y$ ;  $P = 0.005$ ). Reprinted from Karaolis-Danckert et al. (2008) with permission from publisher.

<sup>6</sup> Rapid weight gain was defined as an increase in weight sex- and age-independent SD scores (SDS) > 0.67 between birth and 24 months.

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**Table 2. Summary of studies assessing maternal smoking and offspring adiposity or levels of hormones involved in energy regulation**

Reference	Cohort	Age at Evaluation	Main Findings Related to Adiposity, Leptin, Adiponectin
(Coutant <i>et al.</i> 2001)	Cord blood collected from 87 healthy, full-term newborns at University of Angers, France	birth	<ul style="list-style-type: none"> <li>No difference in log transformed cord leptin (5.9 µg/l in 30 smokers vs 6.0 µg/l in 57 non-smokers)</li> <li>No difference in log transformed maternal serum leptin (15.7 µg/l in 30 smokers vs 17.0 µg/l in 57 non-smokers)</li> </ul>
(Harvey <i>et al.</i> 2007)	448 mother offspring pairs participating in the Southampton Women's Survey (prospective study)	infant	<ul style="list-style-type: none"> <li>Weak trend towards ↓ percent fat mass and ↑ percent lean mass based on DXA scan within 2 weeks of birth [adjβ (SD), percent fat mass = -0.283 (-0.595, 0.029), p=0.075; percent lean mass - 0.257 (-0.059, 0.573), p=0.111]</li> </ul>
(Helland <i>et al.</i> 2001)	609 healthy pregnant women; 19-35 years of age recruited from 2 hospitals in Oslo area Maternal report of smoking during pregnancy (n=209; 76 smokers/133 non-smokers)	birth, 4 & 13 weeks	<ul style="list-style-type: none"> <li>Lower plasma leptin at 13 weeks (median 6.0 µg/L in maternal smoking group versus 3.9 µg/L in non-smoking group; Mann-Whitney p = 0.001)</li> <li>No difference in cord plasma levels (median 6.3 µg/L in maternal smoking group versus 7.0 µg/L in non-smoking group; p = ns)</li> <li>No difference in plasma leptin at 4 weeks (median 4.5µg/L in maternal smoking group versus 3.4 µg/L in non-smoking group; p = ns)</li> </ul>
(Karaolis-Danckert <i>et al.</i> 2008)	370 infants from the German Multicenter Allergy study (prospective study)	2-6 y	<ul style="list-style-type: none"> <li>Rapid growers exposed to tobacco <i>in utero</i> subsequently gained more percent body fat between 2 and 6 y than did rapid growers who had not been exposed (Figure 2) <u>rate of change, 2-6 years (adjβ±SE)</u> time x maternal smoking = 0.14±0.14%/y, p = 0.3 time x maternal smoking x rapid weight gain = 0.78±0.28%/y, p = 0.005</li> </ul>
(Kayemba-Kay's <i>et al.</i> 2008)	Cord serum collected from 1215 Caucasian mothers	birth	<ul style="list-style-type: none"> <li>No relationship between mean cord leptin (ng/ml) and maternal smoking status (never smoked: 8.53±6.90 ng/ml; range in women who smoked &lt;10 cigarettes per day to &gt; 20 per day: 7.63±5.43 to 10.63±11.72)</li> <li>Maternal smoking was considered an important variable in the multiple regression model for cord leptin after adjustment for placenta weight, gender, gestational length, birth weight (p&lt;0.042)</li> <li>No difference in skinfold thickness and mid-arm circumference in newborns in maternal smoking versus non-smoking groups</li> </ul>
(Luciano <i>et al.</i> 1998)	112 newborn infants at the Verona City Hospital, Italy assessed for fat mass and skin fold thickness	newborn	<ul style="list-style-type: none"> <li>Less fat mass and lower anthropometric measures of adiposity (e.g., skin fold thickness) in newborns of mother's who did not smoke but were exposed to passive smoking or who reported light smoking during pregnancy (10 cigarettes per day)</li> </ul>
(Mantzoros <i>et al.</i> 1997)	Cord blood on 62 newborns (50 full-term, 12 premature) delivered by mother's who smoked at the Paras General	birth	<ul style="list-style-type: none"> <li>Lower cord blood leptin associated with maternal smoking during pregnancy. Correlation between cord blood leptin in full-term infants and</li> </ul>

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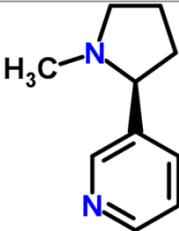
Reference	Cohort	Age at Evaluation	Main Findings Related to Adiposity, Leptin, Adiponectin
	University Hospital, Greece. An additional 50 full-term, 12 premature born to mothers who did not smoke were used as controls		number of cigarettes smoked/d): $r = -0.438$ , $p < 0.001$ ). The effects was more pronounced in premature infants
(Ozkan <i>et al.</i> 2005)	Comparison of leptin levels in Turkish mothers who smoked (n=21) or did not smoke (n=23) during pregnancy and their offspring	7 days	<ul style="list-style-type: none"> <li>• Lower leptin levels in infant serum in maternal smoking group (2.1 versus 2.9 ng/ml in infants of mothers who did not smoke, <math>p = 0.02</math>)</li> <li>• No difference in maternal serum or breast milk leptin</li> </ul>
(Pardo <i>et al.</i> 2004)	Cord blood for 110 term, appropriate-for-gestational age newborns born to mothers who smoked during pregnancy (n=19) or did not smoke (n=91)	birth	<ul style="list-style-type: none"> <li>• No difference in cord blood leptin in infants (7.87 ng/ml in maternal smoking group versus 7.89 ng/ml, <math>p = 0.915</math>)</li> </ul>
(Pardo <i>et al.</i> 2005)	Cord blood for 108 newborns; n=21 in maternal smoking group (14 full-term and 7 preterm newborns) and n=87 in non-smoking group (77 full-term and 10 preterm neonates)	birth	<ul style="list-style-type: none"> <li>• Lower adiponectin levels in premature infants in maternal smoking group (mean of 16.34 <math>\mu\text{g/ml}</math> versus 23.28 <math>\mu\text{g/ml}</math> in infants of mothers who did not smoke; <math>p &lt; 0.05</math>)</li> <li>• No difference in adiponectin levels in full-term infants (mean of 24.18 <math>\mu\text{g/ml}</math> in maternal smoking group versus 25.65 <math>\mu\text{g/ml}</math> in non-smoking group)</li> <li>• Significant correlation between cord blood adiponectin and number of cigarettes smoked/day: <math>r = -0.245</math>, <math>p = 0.01</math></li> </ul>
(Power <i>et al.</i> 2010)	8,815 men and women in a 1958 British cohort (maternal smoking recorded at birth, prospective study)	45 y	<ul style="list-style-type: none"> <li>• Positive association between prenatal maternal smoking and waist circumference  <u>adj mean difference (95% CI) = 1.76 cm (1.20 to 2.33); n=8,493</u>  <u>adj OR (95%CI) for high waist circumference (♀ <math>\geq 88</math> cm; ♂ <math>\geq 102</math> cm) = 1.32 (1.19 to 1.47)</u> </li> </ul>
(Thomas <i>et al.</i> 2007)	7,518 participants of the 1958 British Birth Cohort with information on A1C (prospective study)	45 y	<ul style="list-style-type: none"> <li>• No increased risk for <math>\text{A1C} \geq 6\%</math> after adjustment for birth at gestational age and adult adiposity  <u>adjOR (95%CI), basic model = 1.33 (1.04–1.71)</u>  <u>adjOR (95%CI), basic model + birth weight for gestational age + adult adiposity = 1.01 (0.78–1.32)</u> </li> </ul>
(Vatten <i>et al.</i> 2002)	Umbilical cord plasma collected from 585 singleton infants collected as part of prospective study from 1993-1995 in Stavanger, Norway (maternal smoking at 18 weeks gestation)	birth	<ul style="list-style-type: none"> <li>• No difference in mean cord leptin [mean difference (95% CI) = 0.6ng/ml (-0.9 to 2.0)]</li> </ul>

## Animal Studies of Developmental Exposure to Nicotine

There are 599 known cigarette additives (Rabinoff *et al.* 2007) and most are uncharacterized for potential toxicity. Aside from nicotine, no studies were identified that assessed developmental exposure to ingredients in cigarettes and postnatal effects on glycemic control or adiposity. The studies discussed below are summarized in more detail in [Appendix Table B](#).

### Glucose homeostasis and insulin sensitivity

Several studies from different research groups report hyperinsulinemia and/or impaired insulin tolerance<sup>7</sup> in the male offspring of rats that were treated with nicotine during gestation (Somm *et al.* 2008), lactation (Oliveira *et al.* 2010a), or gestation and lactation (Bruin *et al.* 2007; Bruin *et al.* 2008c; Holloway *et al.* 2005) ([Table 3](#)). [None of these studies included assessment of female offspring]. Administered doses ranged from 1 to 6 mg/kg-day delivered to the mother either via an osmotic mini-pump implanted under the skin or via daily subcutaneous injections<sup>8</sup>. These dosing protocols result in maternal serum cotinine levels that are considered relevant to women who smoke or use nicotine patches as cigarette substitutes.<sup>9</sup> The most consistent findings from these studies were indications of insulin intolerance in adulthood based on either increased insulin area under the curve (AUC) following an oral or ip glucose challenge (Bruin *et al.* 2007; Bruin *et al.* 2008c; Holloway *et al.* 2005; Somm *et al.* 2008) or an increased insulin resistance index (Oliveira *et al.* 2010a). Holloway *et al.*

<b>Nicotine</b>
54-11-5
C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> (MW 162.23)

<b>Use:</b> Alkaloid found in the nightshade family of plants ( <i>Solanaceae</i> ) that constitutes approximately 0.6–3.0% of dry weight of tobacco. Considered the main factor responsible for dependence forming properties for tobacco use
<b>Mechanism:</b> binds to nicotinic acetylcholine receptors (nAChRs)
<b>Assays for nAChRs included in ToxCast™:</b> <a href="#">CHRNA4</a> and <a href="#">Chrna7</a>

<sup>7</sup> Insulin abnormalities can be manifest and described in a variety of ways: hyperinsulinemia = increased fasting insulin; impaired insulin response to a glucose challenge = more or less insulin released to make the glucose come back to normal; and insulin resistance = tissues are resistant to the action of insulin to normalize glucose.

<sup>8</sup> Overall, the evidence is considered more supportive of a direct effect of nicotine rather than effects related to hypoxia/ischemia from using a subcutaneous injection as the route of administration. There is some evidence for transient oxidative stress in the placenta following injections based on increases in antioxidant enzymes by western blotting but no change in heme oxygenase 1 which should be induced by hypoxia. Moreover, other models of placental insufficiency (with profound hypoxia/ischemia) they often find much more significant reductions in birth weight than observed with nicotine.

<sup>9</sup> The dosing protocol used by Somm *et al.* (2008) of treating Sprague Dawley rats via sc osmotic mini-pump at a dose of 3 mg/kg/day results in circulating nicotine metabolite levels measured in gestating females that range from 250 and 300 ng/ml, considered relevant to smokers consuming between 10 and 19 cigarettes/day and pregnant women using nicotine patches as tobacco substitutes (de Weerd *et al.* 2002; Hackman *et al.* 1999). The dose treatment used by Bruin and Holloway (1 mg/kg-day of nicotine bitartrate by sc injection) results in maternal serum cotinine concentrations of 136 ng/ml, which is within the range of cotinine levels (80–163 ng/ml) reported in women who are considered as ‘moderate smokers’ (Bruin *et al.* 2007; Holloway *et al.* 2006).

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(2007) also reported insulin intolerance in 15-week old F2 generation male offspring of dams that were treated with nicotine only during gestation and lactation. Impaired glucose tolerance is also generally observed in studies that conducted glucose tolerance tests (Bruin *et al.* 2007; Bruin *et al.* 2008c; Holloway *et al.* 2005; Somm *et al.* 2008). Effects on fasting insulin levels are somewhat more inconsistent with some studies showing no effect (Bruin *et al.* 2007; Somm *et al.* 2008) and others reporting increased levels in adulthood compared to controls (Holloway *et al.* 2007; Holloway *et al.* 2005; Oliveira *et al.* 2010a). Fasting glucose was the least sensitive endpoint as it was not significantly altered compared to control levels in any study. At lower dose levels of 1 mg/kg-d exposure needed to occur during both fetal and neonatal life (i.e., pregnancy and lactation) to observe permanent changes in glucose homeostasis when higher dose levels used were higher, 3mg/kg-d (Somm *et al.* 2008) or 6mg/kg-d (Oliveira *et al.* 2010a), then either fetal or lactational exposure was sufficient to see changes.

Two studies have looked at the impact of cigarette smoke on glycemic control in pregnant Wistar rats (de Souza Mda *et al.* 2009; Sinzato *et al.* 2008). Neither of these studies included long-term assessment of offspring, but both presented data on blood glucose in dams during pregnancy. Dams were either non-diabetic or made diabetic prior to pregnancy by treatment with 40 mg/kg streptozotocin (STZ) 7 days before mating. Sinzato *et al.* (2008) studied rats (n=10/group) that were either exposed to cigarette smoke<sup>10</sup> before pregnancy or before and during pregnancy. Blood glucose was measured in dams on GD0 and GD21 and liver glycogen on GD21. Exposure to cigarette smoke in non-diabetic animals did not alter blood glucose compared to unexposed controls regardless of whether the exposure was prior to pregnancy or before and during gestation. Cigarette smoke seemed to exacerbate the diabetic effect of STZ at GD21 (there were no statistically significant differences between treatment groups on GD1). On GD21, the average blood glucose level in diabetic animals not exposed to cigarette smoke was  $471.8 \pm 21.9$  mg/dL. The levels of blood glucose in diabetic animals exposed to cigarette smoke were statistically higher:  $525.1 \pm 25.7$  mg/dL in animals exposed to cigarette smoke before pregnancy and  $562.1 \pm 19.9$  mg/dL in animals exposed before and during pregnancy. Liver glycogen levels were decreased in non-diabetic animals exposed to cigarette smoke before or before and during pregnancy ( $4.2 \pm 0.2$  mg/100mg versus  $2.4 \pm 0.4$  and  $3.2 \pm 0.5$  mg/100 mg, respectively). In diabetic animals, liver glycogen was lower in animals that were exposed to cigarette smoke before and during pregnancy compared to controls ( $2.6 \pm 0.3$  versus  $1.9 \pm 0.4$  mg/100 mg), but this difference was not statistically significant. A second study by this research group, de Souza Mda, (2009), focused on pregnancy outcome in the rats and also included data on blood glucose in dams during pregnancy. Based on comparing the blood

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<sup>10</sup> The composition of the cigarette used consisted of 10 mg of tar, 0.80 mg of nicotine and 10 mg of carbon monoxide. For exposure to tobacco cigarette smoke, ~47-day-old rats were placed in hermetically sealed chambers before pregnancy while others were exposed before and during pregnancy period. Control groups were exposed to filtered air during similar periods of time. In the first week of exposure, non-pregnant rats were submitted to an adaptation period and exposed to smoke using 5 cigarettes for 30 minutes/day during seven days. After adaptation, rats were exposed to smoke from 10 cigarettes for 30 minutes on a daily basis with 15-minute resting intervals for release of all cigarette smoke contained in the chamber. Following the experimental procedure, the same animals were exposed to smoke from another 10 cigarettes for 30 more minutes; this proceeding method was used for approximately two months (Sinzato *et al.* 2008).

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glucose levels between the 2 papers, it seems likely that the glucose data is based on the same experimental animals.

The developmental effects of nicotine described above are not seen when nicotine treatment starts later. Several studies by Swislocki et al. (2003; 2008; 1997) using older animals, male and female Sprague-Dawley rats ranging in age from 4 weeks to adulthood, did not report indications of effects on glucose, insulin or leptin based on fasting measurements or response during oral glucose tolerance testing. This may identify gestation and lactation as a critical period. There are also differences in experimental protocol that could contribute to the different findings. These researchers used 50 mg nicotine pellet implants to administer nicotine and internal dosimetry was not characterized. Thus it is unknown whether differences in route of administration could have resulted in differences in nicotine delivery and contributed to the discrepant results (i.e., did the pellet result in an initial surge of nicotine levels followed by low levels versus mini-pump studies which deliver a more consistent amount of nicotine?). In any case, the amount of administered nicotine differed in male and female rats on a body weight basis because the male animals were larger (190 versus 160 g) and this was not considered in the statistical analysis using a 2 way ANOVA. Similarly, no effect on plasma glucose levels was reported by Jose et al. (2009) in a study of 30-day old male Wistar rats treated with 5 mg/kg-day nicotine for 28 or 56 days by sc injection. However, based on the findings described above for fasting glucose, a lack of an effect on non-fasted plasma glucose levels in Jose et al. (2009) is difficult to interpret as a “negative” given the apparent insensitivity of this endpoint.

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**Table 3. Summary of study findings related to glucose homeostasis and insulin sensitivity following developmental exposure to nicotine**

Study Design	Reference	Endpoint	Effect
<i>gestation</i>			
3 mg/kg-day; Sprague-Dawley rats treated during GD4-GD17 by sc osmotic mini-pump (5-10 F1 ♂/group, number of dams not reported)	Somm et al. (2008) *data from F1 ♂ at 26 weeks of age	fasting serum glucose (mmol/l)	↔
		fasting insulin (ng/ml)	↔
		glucose, ipGTT	↑ AUC
		insulin, ipGTT	↑ (decreased insulin sensitivity)
		glucose, ITT	↑ AUC
<i>lactation</i>			
6 mg/kg-day; Wistar rats treated during PND3-PND16 by sc osmotic mini-pump (6 dams per group/36 F1 ♂)	Oliveira et al. (2010a)	fasting serum glucose (mg/dl)	↔
		fasting insulin (μUI/ml)	↑
		insulin resistance index (fasting insulin x fasting glucose)	↑
<i>gestation + lactation</i>			
1 mg/kg-day; Wistar rats treated 2 weeks prior to mating until weaning by sc injection (sample sizes for individual studies in <a href="#">Appendix Table B</a> ).	Holloway et al. (2005)	fasting serum glucose (mmol/l)	↔ PND1 or 26 weeks
		fasting insulin (ng/ml)	↓ PND1, ↑ at 26 weeks
		glucose, OGTT	↑ AUC at 7 and 26 weeks
		insulin, OGTT	↑ AUC at 26 weeks
	Holloway et al. (2007) *data from adult F2 ♂ assessed at 15 weeks of age	fasting serum glucose (mmol/l)	↔
		fasting insulin (ng/ml)	↑
		glucose AUC, IPGGT	↔
		insulin AUC, IPGGT	↑
	Bruin et al. (2007) *data from adult F1 ♂ assessed at 26 weeks of age	fasting serum glucose (mmol/l)	↔
		fasting insulin (ng/ml)	↔
		insulin:glucose	↔
		glucose AUC, OGGT	↑
Bruin et al. (2008c)	insulin AUC, OGGT	↑	
	glucose AUC, OGGT	↔ at 4 weeks; ↑ at 15 and 26 weeks	

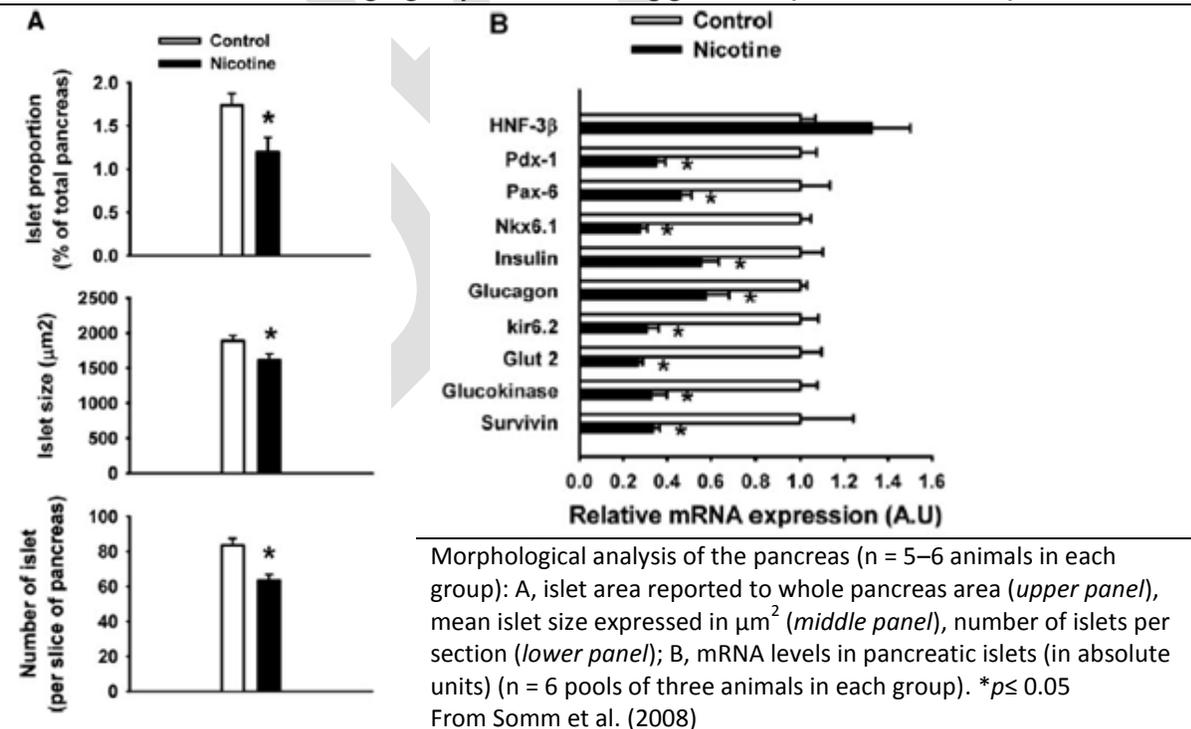
↔ = no effect; ↑ or ↓ = statistically significant increase or decrease ( $p \leq 0.05$ )

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*Pancreatic effects*

Changes in pancreatic weight, morphology and function have been reported in animals that were treated with nicotine during gestation or gestation and lactation (Bruin *et al.* 2008a; Bruin *et al.* 2007; Bruin *et al.* 2008b; Grove *et al.* 2001; Holloway *et al.* 2005; Somm *et al.* 2008). The types of effects reported include  $\beta$ -cell or islet cell apoptosis and decreased  $\beta$ -cell mass in the male F1 offspring of Wistar rats treated with 1 mg/kg-day nicotine bitartrate by sc injection from 2-weeks prior to mating to weaning (Bruin *et al.* 2007; Holloway *et al.* 2005). A similar finding of decreased islet cell mass was reported by Somm *et al.* (2008) in male PND7 Sprague Dawley (OFA strain) offspring of dams treated with 3 mg/kg-day of nicotine hydrogen tartrate by subcutaneous osmotic mini pump infusion from GD4 to GD17 (Figure 3). As noted earlier, the treatment protocols used in these rodent studies result in maternal serum cotinine levels that are considered relevant to women who smoke or use nicotine patches as cigarette substitutes. Grove *et al.* (2001) reported a significant decrease in the weight of the pancreas to 65% of control values in infant Rhesus monkeys whose mothers were treated with 1.5 mg/kg-day nicotine tartrate by subcutaneous osmotic mini pump infusion from GD26 to near term on GD160. The pancreatic effects are hypothesized to occur as a direct effect from nicotine binding to nicotinic acetylcholine receptors located in the developing pancreas, leading to oxidative stress and mitochondrial damage (discussed below) and eventual  $\beta$ -cell apoptosis (Bruin *et al.* 2008b). Pancreatic tissue is considered to be especially susceptible to oxidative-stress mediated tissue damage due to low levels of antioxidant enzyme expression (Lenzen *et al.* 1996; Tiedge *et al.* 1997).

**Figure 3. Altered pancreatic effects in male Sprague Dawley rats pups on PND7 following maternal treatment with 3 mg/kg-day nicotine during gestation (Somm *et al.* 2008)**



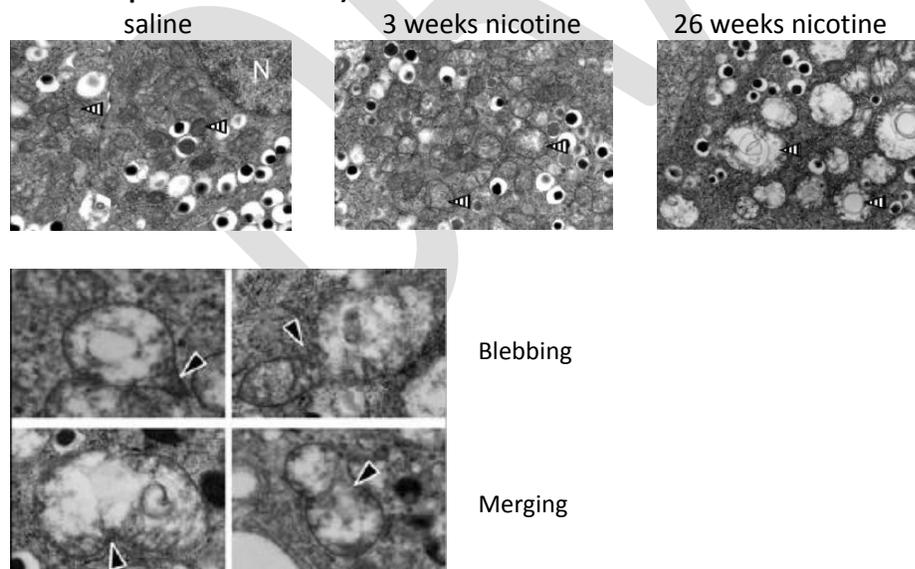
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The effects on pancreas and glycemic regulation have been at least partly attributed to mitochondrial dysfunction reflected by morphological observations of mitochondrial swelling, blebbing, or merging (Bruin *et al.* 2008a; Bruin *et al.* 2007; Bruin *et al.* 2008b; Bruin *et al.* 2008c) (Figure 4). Bruin *et al.* (2008c) conducted post-natal assessments of pancreatic mitochondria and OGTT at 3 or 4 weeks, 15 weeks, and 26 weeks of age in male WistarF1 rats whose dams were treated with 1 mg/kg-day nicotine bitartrate by sc injection from 2-weeks prior to mating to weaning (Table 4). They found indications of mitochondrial structural abnormalities beginning at 3 weeks that worsened with age. Other effects observed at 26 weeks included reduced pancreatic respiratory chain enzyme activity, degranulation of beta cells, and impaired glucose-stimulated insulin secretion compared to saline controls. Certain morphological effects in the mitochondria, namely that a reduction in the percent of mitochondria considered

**Table 4. Postnatal assessment of mitochondria,  $\beta$ -cells, and glucose tolerance of male Wistar rats following maternal treatment with nicotine prior to mating, during gestation, and until weaning on PND21 (Bruin *et al.* 2008 an open access article.)**

3 weeks	↓ percentage of mitochondria considered structurally intact ("stage 1")
15 weeks	impaired glucose tolerance; ↓ percentage of mitochondria considered structurally intact ("stage 1"); ↑ mitochondrial area and % of mitochondria with blebbing and/or merging; ↓ total and immature number of insulin granules/ $\beta$ cell area
26 weeks	impaired glucose tolerance; ↑ mitochondrial area and % of mitochondria with blebbing and/or merging; ↓ percentage of mitochondria considered structurally intact ("stage 1"); ↓ total and immature number of insulin granules/ $\beta$ cell area

**Figure 4. Electron microscopy of mitochondrial morphology (Bruin *et al.* 2008 an open access article)**



structurally healthy ("stage 1") preceded the development of impaired OGTT that was first observed at 15 weeks of age (Table 4). The  $\beta$ -cell apoptosis reported above has been attributed to the mitochondrial apoptotic pathway (intrinsic) rather than apoptosis through a death receptor (extrinsic, e.g., Fas) pathway (Bruin *et al.* 2008a). Oxidative stress is also apparent in

pancreatic tissue of these rats as indicated by increased ROS production from islet cells isolated from the nicotine-treated animals at 3 or 26 weeks of age (Bruin *et al.* 2008b; Bruin *et al.* 2008c).

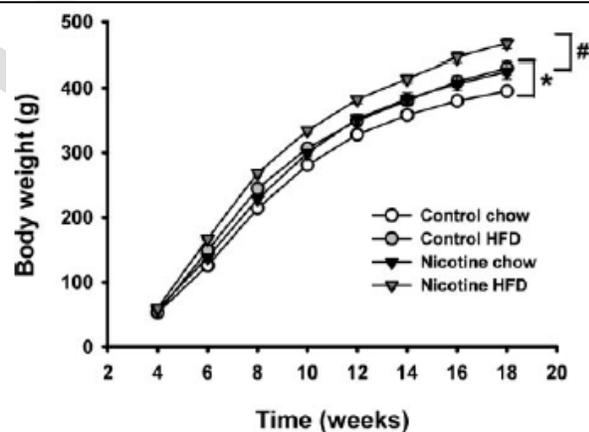
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### *Body weight, adiposity, and measures related to metabolic syndrome*

Body weight in rats exposed to 0.75 to 6 mg/kg-day nicotine during development is generally found to be higher compared to controls with the effect typically first apparent at weaning and persisting through adulthood (Gao *et al.* 2005; Newman *et al.* 1999; Oliveira *et al.* 2010a; Oliveira *et al.* 2009; Somm *et al.* 2008)(Table 5). There are exceptions to this pattern. For example, a study by Gao *et al.* (Gao *et al.*) did not find effects on growth using the same dosing paradigm found to increase adult body weight in other studies by this research group. No effects on body weight in the F2 generation were observed in the transgenerational study of Holloway *et al.* (2007), although the animals did have significant changes compared to controls for serum leptin ( $\uparrow$ ), serum adiponectin ( $\downarrow$ ), and total cholesterol ( $\uparrow$ ). Not all of the studies reporting increased body weight in adulthood included an assessment of food intake, but it was unaffected in studies that did report this endpoint (Oliveira *et al.* 2010a; Oliveira *et al.* 2009; Somm *et al.* 2008). The overall pattern of increased growth later in life is not apparent at birth. Studies that included gestational treatment with nicotine report either no effect on birth weight (Bruin *et al.* 2007; Gao *et al.* 2005; Grove *et al.* 2001; Somm *et al.* 2008; Williams and Kanagasabai 1984) or a decrease in weight compared to controls (Chen *et al.* 2007; Gruslin *et al.* 2009; Holloway *et al.* 2005; Newman *et al.* 1999).

Somm *et al.* (2008) conducted additional studies to assess the impact of consumption of a high fat diet (HFD) in Sprague-Dawley rats that were prenatally exposed to nicotine by comparing growth in animals fed a normal chow or high fat diet (HFD) for 14 weeks, starting at 4 weeks of age. The nicotine treated animals fed a HFD were heavier than control animals consuming the same diet (Figure 5). The nicotine animals did not consume more HFD (6530 kcal/14 weeks versus 6783 kcal/14 weeks in control) but had a significantly increased food efficiency, i.e., they needed less food to gain weight, compared to controls (3.76 versus 4.20 g food/g bw gain). Physical activity was reduced in the nicotine treated animals. During the dark phase when rodents are most active the nicotine treated animals had a significant reduction in spontaneous ambulatory activity compared to controls (10,549 versus 13,944 counts;  $p = 0.04$ ); a similar trend was apparent during lights on phase but it did not reach statistical significance (3,748 versus 4,772 counts;  $p = 0.06$ ). No difference in oxygen consumption or respiratory exchange ratio suggested the effects of food efficiency were not due to decreased energy expenditure.

**Figure 5. Effects on growth in male Sprague Dawley treated with nicotine during gestation (Somm *et al.* 2008)**



From Figure 5 of Somm *et al.* (2008)

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More detailed analysis of leptin levels and adiposity (e.g., fat mass, adipocyte area) show that increases in body weight during postnatal life can be accompanied by increased serum leptin (Oliveira *et al.* 2010a; Oliveira *et al.* 2009) and increased fat mass (Gao *et al.* 2005; Oliveira *et al.* 2010a; Oliveira *et al.* 2009) or adipocyte area (Somm *et al.* 2008). There is little indication of a consistent effect on serum lipids based on the key studies identified for the current review (Table 5). One study looked at adiposity in fetal animals that were exposed to nicotine *in utero*. Williams *et al.* (1984) treated Sprague-Dawley dams with ~2.46 mg/kg-day nicotine tartrate in drinking water from GD0-GD21. The nicotine-treated fetuses had significantly more body fat compared to control animals, 44 versus 29.2 mg/fetus. This finding is inconsistent with the results from other studies showing no effect or a decrease in birth weight; however, body weight can be a crude indicator of adiposity in rodents so it may not be possible to reconcile the fetal adiposity findings with the broader literature. Williams *et al.* (1984) also reported increased lipolysis and transport of fatty acids from maternal adipose tissue into circulation (assessed in *ex vivo* studies measuring glycerol release under basal and stimulated conditions, 1.7- and 1.32-fold respectively). They suggested an increased release of maternal fatty acids could be a contributing factor to the increased adiposity observed in fetuses, but the study did not assess whether the maternally-produced fatty acids crossed the placenta.

In addition to the effects described above, other studies have reported a range of cardiovascular effects in animals treated with nicotine during development including findings of alterations in vascular structure, aortic contractility, and cardiac-related neurotransmission [reviewed in Somm *et al.* (2009) and Bruin *et al.* (2010)]. Some studies include assessment of endpoints related to metabolic syndrome, namely blood pressure and serum lipids. The reviews described epidemiological studies of smoking during pregnancy and increased risk of blood pressure in offspring (Beratis *et al.* 1996; Blake *et al.* 2000; Huang *et al.* 2007; Morley *et al.* 1995) and findings in laboratory animals of prenatal nicotine exposure and exacerbation of increased blood pressure in spontaneously hypertensive rats (Pausova *et al.* 2003) and increased Ang II-stimulated blood pressure in male but not in female adult rats (Xiao *et al.* 2008). These changes have been suggested to be due to endothelial dysfunction (Xiao *et al.* 2007), changes in renal structure (Pausova *et al.* 2003), or changes in the composition and contractile response of perivascular adipose tissue (Gao *et al.* 2008; Gao *et al.* 2005). One study reported increased systolic, diastolic, and mean arterial pressure in 13-week old F2 generation male offspring of dams that were treated with nicotine only during gestation and lactation (i.e., the F2 generation males were not exposed to nicotine *in utero*) (Holloway *et al.* 2007). Other studies have reported persistent loss of responsiveness to sympathetic activation of beta-adrenergic receptors and promotion of parasympathetic muscarinic function following developmental exposure in rats. These effects would serve to reduce cardiovascular work potential and impair energy utilization (Navarro *et al.* 1990; Slotkin *et al.* 1999).

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**Table 5. Summary of study findings related to body weight, adiposity, and serum lipids following developmental exposure to nicotine**

Study Design	Reference	Endpoint	Effect
<i>gestation</i>			
2 mg/kg-day, Sprague-Dawley rats (10 dams/group treated during GD4-GD20 by sc osmotic mini-pump)	Levin et al. (2005; 1996)	birth weight (g)	↔
		body weight (g), PND8-PND29	↑ (~1.05 to 1.09-fold)
		body weight (g), PND35 or PND 43 (food restriction)	↔
3 mg/kg-day; Sprague-Dawley rats treated during GD4-GD17 by sc osmotic mini-pump (5-10 F1 ♂/group, number of dams not reported)	Somm et al. (2008)	birth weight (g)	↔
		body weight (g), weaning	↑
		body weight (g), 18-20 weeks	↑
		eWAT (% of body weight)	↑
		eWAT adipocyte area (μm <sup>2</sup> )	↑
		BAT (% of body weight)	↓
~2.46 mg/kg-day, Sprague-Dawley rats (12 dams/group treated during GD0-GD20 via drinking water)	Williams and Kanagasabai(1984)	fetal body weight (g)	↔
		fetal body fat (mg/fetus)	↑
1.5 mg/kg-day, Rhesus monkeys (6-7 dams/group treated during GD26 – GD160 by sc osmotic mini pump)	Grove et al. (2001)	birth weight	↔
		leptin (ng/ml), PND 1	↓
<i>lactation</i>			
6 mg/kg-day; Wistar dams treated during PND3-PND16 by sc osmotic mini-pump (sample sizes for individual studies in <a href="#">Appendix Table B</a> .)	Oliveira et al. (2010b)	body weight (g), during lactation	↔
		total fat content (%), PND15 and PND21	↑ PND15, ↔ PND21
		visceral fat mass (%), PND15 and PND21	↔
		total protein content (%), PND15 and PND21	↔ PND15, ↑ PND21
		maternal serum leptin (ng/ml), PND21	↑
		milk leptin (ng/ml), PND21	↑
		pup serum leptin (ng/ml), PND21	↔
		total cholesterol (mg/dl), PND15 and PND21	↔
		HDL cholesterol (mg/dl), PND15 and PND21	↑ PND15, ↔ PND21
		LDL cholesterol (mg/dl), PND15 and PND21	↔
		VLDL cholesterol (mg/dl), PND15 and PND21	↔
		triglycerides (mg/dl), PND15 and PND21	↔
		Castelli index I (total C/HDL-C), PND15 and PND21	↔
		Castelli index II (LDL-C/HDL-C), PND15 and PND21	↔
Oliveira et al. (2009) *data from adult F1 ♂		body weight gain (g), after weaning	↑ PND75-100, 165-180
		total body fat (g/100g bw)	↑

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**Table 5. Summary of study findings related to body weight, adiposity, and serum lipids following developmental exposure to nicotine**

Study Design	Reference	Endpoint	Effect
	assessed at PND180 unless stated otherwise	visceral fat mass (g/100g bw)	↔
		body protein (g/100g bw)	↔
		leptin (ng/ml)	↑
Oliveira et al. (2010a) *data from adult F1 ♂ assessed at PND180; the serum lipid data was also presented in Oliveira et al. (2009)		body weight (g), PND180	↑
		Lee's index of obesity,	↑
		food intake(g)	↔
		total fat (cm <sup>2</sup> )	↑
		central fat (cm <sup>2</sup> )	↑
		subcutaneous fat (cm <sup>2</sup> )	↔
		epididymal adipocyte area (μm <sup>2</sup> )	↑
		inguinal adipocyte area (μm <sup>2</sup> )	↑
		leptin (ng/ml)	↑
		adiponectin (μg/ml)	↔
		ratio of adiponectin (μg/ml):visceral fat mass (g)	↓
		ratio of serum leptin:adiponectin	↑
		total cholesterol (mg/dl)	↔
		HDL cholesterol (mg/dl)	↔
		LDL cholesterol (mg/dl)	↔
		VLDL cholesterol (mg/dl)	↔
		triglycerides (mg/dl)	↔
		Apo A1 (mg/dl)	↔
		Apo B1 (mg/dl)	↔
liver glycogen (mM/g)	↓		
muscle glycogen (μM/ml g)	↑		
6 mg/kg-day; Wistar dams treated during PND3-PND16 by sc osmotic mini-pump	Santos-Silva et al. (2010)	body weight, PND15	↔
		body weight, PND180	↑ (1.10-fold)
		visceral fat mass (g/100 g BW), PND15 and 180	↑ (1.8 and 1.27-fold)
		leptin (ng/ml), PND15 and 180	↑
<i>gestation + lactation</i>			
0.75, 1.5, or 3.0 mg/kg-day	Newman et al. (1999)	birth weight	↓
		body weight, PND7 and 14	↑
1 mg/kg-day; Wistar rats treated 2 weeks prior to mating until weaning by sc injection (sample sizes for	Gao et al. (2005)	birth weight (g)	↔
		body weight (g), weaning and beyond	↑
		body weight (g), 26 weeks	↑

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**Table 5. Summary of study findings related to body weight, adiposity, and serum lipids following developmental exposure to nicotine**

Study Design	Reference	Endpoint	Effect
individual studies in <a href="#">Appendix Table B</a> .		fat pad (g)	
		total	↑
		epididymal	↑
		mesentery	↑
		perirenal	↑
	Holloway et al. (2005)	birth weight	↓
Holloway et al. (2007) F2 generation effects		body weight (g), weaning	↓
		body weight (g), 15 weeks	↔
		fat pad weight (% total body weight)	↔
		leptin (ng/ml)	↑
		adiponectin (ng/ml)	↓
		total cholesterol (mg/dl)	↑
		triglycerides (mmol/l)	↔
		NEFA (mEq/l)	↔
	Bruin et al. (2007)	birth weight	↔
Gao et al. (2008)		body weight (g), 26 weeks	↔
		visceral white fat (g)	↔
		interscapular brown fat (g)	↔
		body weight (g), 26 weeks	↔
Gruslin et al. (2009)		fetal weight, (GD21)	↓
		birth weight (PND1)	↓
		body weight at weaning (PND21)	↔

↔ = no effect; ↑ or ↓ = statistically significant increase or decrease ( $p \leq 0.05$ )

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*Leptin, thyroid, and adrenal*

[Leptin](#) is a key hormone involved in feeding behavior and energy expenditure [reviewed in Belgardt et al. (2010)]. In general terms, leptin is secreted from the adipocytes of white adipose tissue and delivers information on peripheral energy stores to the central nervous system. Several studies have looked at the effect of nicotine on leptin levels or expression levels of proteins involved in leptin signaling following developmental exposure (Grove *et al.* 2001; Huang and Winzer-Serhan 2007; Santos-Silva *et al.* 2010). Grove et al. (2001) reported 50% lower levels of leptin in neonatal Rhesus monkeys following maternal treatment with 1.5 mg/kg-d nicotine during pregnancy (Table 6). This finding is consistent with the studies of newborn human infants exposed to maternal smoking described above to the extent that serum samples taken directly from a neonate can be compared with cord blood levels at delivery. The human studies reported no difference in leptin or a decrease in infants whose mothers smoked during pregnancy (Table 2).

**Table 6. Effects neonatal Rhesus monkeys following maternal treatment with 1.5 mg/kg-day nicotine tartrate during pregnancy (Grove et al. 2001)**

	saline	nicotine
pancreas weight (g)	0.40 ± 0.04	0.26 ± 0.02*
adrenal weight (g)	0.25 ± 0.03	0.19 ± 0.02*
leptin (ng/ml) <sup>1</sup> ; serum on PND1	~7.5	~3.75*
cortisol (ng/ml) <sup>2</sup> ; serum on PND1	~375	~280
NPY mRNA in ARH (normalized silver grain area)	1.0	~0.6*
POMC mRNA in ARH (normalized silver grain area)	1.0	~2.0*
birth weight (g)	436.8 ± 11	423.0 ± 6.3
birth weight normalized to maternal body weight [birth weight (g)/maternal weight (kg)]	64.1 ± 2.9	57.6 ± 3.9

From Grove et al. (2001)

\* $p \leq 0.05$

<sup>1</sup>No effect on leptin levels in amniotic fluid measured on GD118, 130, or 160.

<sup>2</sup>Cortisol levels in amniotic fluid were significantly decreased on GD118 and 160, but not on GD130

The other two studies used rats as the experimental animal model (Huang and Winzer-Serhan 2007; Santos-Silva *et al.* 2010). Both found significant elevations in leptin levels, although the studies reported opposite effects on body weight. Both studies also reported changes in neural tissue expression levels of proteins involved in leptin signaling. Huang et al. (2007) treated neonatal Sprague-Dawley rats with 0, 0.25, 1.5, or 3 mg/kg nicotine twice a day for seven days (0, 0.5, 3, and 6 mg/kg-d) and once on PND8 by oral gavage and then assessed body weight, serum leptin and mRNA expression of several peptides related to feeding behavior (NPY, AgRP, POMC, CART<sup>11</sup>) in hypothalamic arcuate nucleus (Arc). This treatment regimen led to significant dose-dependent decreases in body weight in all dose groups (7-20% reductions) and significant increases in serum leptin in the 3 and 6 mg/kg-d groups (1.66 and 1.9-fold, respectively). The

<sup>11</sup> NPY = neuropeptide Y; AgRP = agouti-related protein; POMC = proopiomelanocortin (POMC); CART = cocaine- and amphetamine-regulated transcript

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number of heteromeric  $\alpha 4\beta 2^*$  nicotinic acetylcholine receptors (nAChRs) were increased in both the Arc and ventromedial nucleus based on binding studies with  $^{125}\text{I}$ -epibatidine. Expression of NPY, AgRP, POMC mRNA were increased in the Arc in all dose groups. Co-administration to animals treated with 6 mg/kg-d nicotine with the  $\alpha 4\beta 2^*$  nAChR antagonist dihydro-b-erythroidine (DH $\beta$ E) did not attenuate the effects on body weight, partially suppressed the elevated serum leptin, and completely blocked the mRNA expression changes.

Santos-Silva et al. (2010) looked at the effect of lactational exposure to nicotine on serum leptin and leptin signaling in the pituitary and thyroid gland on PND15 or 180. Dams were implanted with an sc osmotic mini-pump designed to deliver 6 mg/kg-d nicotine from lactation day 2 for 14 days and animals were collected on PND15 or PND180. The amount of visceral fat mass and leptin levels was significantly increased at both time points; body weight was elevated as well, but the effect was only statistically significant at PND180 (Table 5). There was no difference in food intake during development. Serum levels of thyroid hormone T3 and T4 were significantly decreased and TSH was increased at both ages. Levels of several proteins involved in leptin signaling were altered in the pituitary, including leptin receptor b or OB-Rb, JAK-2, and STAT-3.<sup>12</sup> Fewer protein expression changes were seen in the pituitary compared to the hypothalamus or thyroid gland. Fewer changes in thyroid expression levels were seen in older animals compared to the younger animals. The patterns of response were not necessarily consistent between tissues or age of assessment on PND15 and PND180. In particular, hypothalamic expression of OB-R was significantly increased on PND 15 (1.58-fold) but decreased on PND180 (39% of control). At both time points the direction of effect in the hypothalamus was opposite the direction of effect in the thyroid (see study summary in Appendix Table B for complete description of direction and magnitude of effects).

Thyroid effects resulting from treatment with nicotine during development were also identified in Oliveira et al. (2009). On PND15 the animals had decreased levels of free triiodothyronine (FT<sub>3</sub>) and thyroxine (FT<sub>4</sub>) and higher thyroid stimulating hormone (TSH). The effects on FT<sub>3</sub> and FT<sub>4</sub> were also observed at 180 days, although the direction of effect on TSH was opposite, i.e., it was decreased relative to control values. At both time points activity of liver type 1 deiodinase was lower (26% at 15 days and 55% at 180 days).

Developmental exposure to nicotine has been shown to adrenal axis function and responsiveness (Grove et al. 2001; Navarro et al. 1990; Navarro et al. 1988; Oliveira et al. 2010b; Oncken et al. 2003; Sarasin et al. 2003; Slotkin et al. 1995; von Ziegler et al. 1991). Oliveira et al. (2010b) found increases in serum levels of corticosterone and adrenal catecholamine content of ~1.5-fold and 1.70-fold higher, respectively, on PND15 in rats whose dams were implanted with an sc mini-pump set to administer 6 mg/kg-day of nicotine during lactation. Adrenal gland weight was also increased in these animals by ~1.40-fold and levels of [tyrosine hydroxylase](#) (TH) in the adrenal medulla were decreased to ~66% of control. The effects on corticosterone, catecholamine and adrenal weight were unaffected at the time of

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<sup>12</sup> OB-R = leptin receptor, JAK-2 = Janus tyrosine kinase 2; STAT-3 = Signal transducer and activator of transcription 3; pSTAT-3 = phosphorylated (activated) STAT1

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weaning on PND21 (TH was not assessed at this time point) (Oliveira *et al.* 2010b). Maternal corticosterone levels and adrenal catecholamine content were unaffected at these time points. Another study from this research group showed no effect on corticosterone levels in F1 males at 180 days following the same treatment protocol early in life (Oliveira *et al.* 2010a). von Ziegler *et al.* (1991) also reported increased plasma corticosterone in fetal (GD18) male Long Evans rats whose dams were implanted with an osmotic mini-pump set to administer 6 mg/kg-d nicotine tartrate for 1 week beginning on GD12. The effect was no longer apparent by PND15 and not observed in dams or female offspring at any age. Grove *et al.* (2001) found decreased adrenal weight but no difference in cortisol levels of infant monkeys of mothers treated with nicotine during pregnancy. In piglets (5-6/group), small to moderate doses of nicotine (0.130 or 0.260 mg/kg-h by iv infusion) did not cause an increase in plasma catecholamines whereas the highest dose tested, 1 mg/kg-h, caused a significant increase in adrenaline (Andresen *et al.* 2008). Cord blood levels of epinephrine and norepinephrine are also lower in infants of mothers who smoked during pregnancy (Oncken *et al.* 2003). The effects on the adrenal axis from developmental exposure to nicotine have been attributed to an impaired ability to activate the sympatho-adrenal axis resulting in compromise of adaptive responses to metabolic challenges or hypoxia (Navarro *et al.* 1990; Navarro *et al.* 1988; Slotkin *et al.* 1995). Some of these findings have been attributed to a reduced ability of adrenomedullary chromaffin cells to respond to asphyxia stressors, an adaptive response that appears to be mediated by direct actions of nicotine on nicotinic acetylcholine receptors (Buttigieg *et al.* 2008).

**Appendix Tables**  
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**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

Study Design	Population	Exposure Assessment	Outcome (prevalence)	Risk Estimate (95% CI)	Adjusted For
retrospective cohort US, Wisconsin (Adams <i>et al.</i> 2005)	252 American Indian mother-child pairs in the WIC program, ≥ 36 week gestation with no birth defects affecting growth, and with data on maternal smoking, child birth weight, and child height and weight at 36 months (46.8% boys)	Maternal report of smoking during pregnancy at initial WIC visit (42.5%)  Overweight = BMI ≥95 <sup>th</sup> percentile (22% prevalence)  At risk of overweight = BMI ≥85 <sup>th</sup> to <95 <sup>th</sup> percentile (18.7% prevalence)	Overweight at 3 years: BMI ≥ 85 <sup>th</sup> percentile [n= 252; 103 with BMI ≥ 85 <sup>th</sup> (40.9%)/149 BMI <85 <sup>th</sup> percentile ]	<b>adjOR = 2.16 (1.05-4.47)</b> crudeOR = 1.74(1.05-2.90) <sup>1</sup>	<u>Mother</u> : age, BMI, education, income <u>Child</u> : birth weight, ever breastfed
prospective cohort Australia, Queensland (Al Mamun <i>et al.</i> 2006)	3,253 subjects in the Mater University Study of Pregnancy and its Outcomes (n of total cohort = 7,223; n in OR analysis 2,728)	Maternal report of smoking during pregnancy (36.6%)	Overweight at 14 years: 85 <sup>th</sup> ≤ BMI < 95 <sup>th</sup> percentile (19.3% prevalence)  Obese at 14 years: BMI ≥ 95 <sup>th</sup> percentile (6.2% prevalence)	<b>adjOR = 1.30 (1.05-1.60)</b> crudeOR = 1.30 (1.07-1.58)  <b>adjOR = 1.40 (1.01-1.94)</b> crudeOR=1.41 (1.04-1.91)	<u>Mother</u> : age, marital status, income <u>Child</u> : age, sex, breastfeeding, consumption of fast food, salad, soft drinks, red meat, TV viewing, participation in sports and exercise
prospective cohort Berlin, Dusseldorf, Freiburg, Mainz and Munich, Germany (Bergmann <i>et al.</i> 2003)	1314 singleton term infants born during 1990 as part of the German Multicenter Atopy Study that included six delivery units (52.8% boys).  918 children could be followed until age 6, but only 480 cases with complete data for BMI, triceps and subscapular skin fold were included in risk estimate analyses.	Maternal reported smoking during pregnancy (19.6%; not reported how assessed)	Overweight at 6 years: BMI ≥90 <sup>th</sup> to < 97 <sup>th</sup> percentile (n=480; 9.5% prevalence) <sup>1</sup>  Obese: BMI ≥ 97 <sup>th</sup> percentile (9.5% prevalence in the 480 cases <sup>1</sup> )	<b>adjOR= 2.08 (1.19-3.63)</b> crudeOR=1.78 (1.21-2.64)  <b>adjOR= 2.30 (1.15-4.60)</b>	<u>Mother</u> : overweight in pregnancy, social status <u>Child</u> : breastfeeding
prospective cohort US, multi-site (Chen <i>et al.</i> 2006)	34,866 children enrolled in Collaborative Perinatal Project in 1959-1965; ~50% boys; evaluated at 1, 3, 4, 7, and 8 years of age.  Risk estimate analysis is based on n=6,181 boys at age 8 and n=6,235 girls at age 8	Maternal report of smoking during pregnancy (51.4%) current smoker vs. never smoker	<b>Overweight or obese at 8 years: BMI ≥ 85<sup>th</sup> percentile (11%<sup>1</sup>)</b> boys (n=6,181)  girls (n=6,235)  <b>Obese at 8 years: BMI ≥ 95<sup>th</sup> percentile (4%<sup>1</sup>)</b> boys (n=6,181)  girls (n=6,235)	<b>adjOR = 1.21 (1.05-1.39)</b> crudeOR = 1.23 (1.08-1.39) <sup>1</sup>  <b>adjOR = 1.37 (1.19-1.58)</b> crudeOR = 1.29 (1.14-1.47) <sup>1</sup>  adjOR = 1.21 (0.96-1.51) crudeOR = 1.17 (0.96-1.43) <sup>1</sup>  adjOR = 1.31 (1.06-1.61) crudeOR = 1.20 (0.99-1.44) <sup>1</sup>	<u>Mother</u> : age, race, socioeconomic status, pre-pregnancy BMI, marital status, gestational age at recruitment, recruitment site <u>Child</u> : age, birth order, breastfeeding
prospective cohort Canada, Quebec (Dubois and Girard 2006)	2,103 children born in Quebec in 1998 participating in the Quebec Longitudinal Study Child Development from birth through 4.5 years (51% boys)  Measured height and weight data available for 1,550 children at 4.5 years of age	Maternal report of smoking during pregnancy assessed 5 months post-partum (25.2%)	BMI ≥ 95 <sup>th</sup> percentile at 4.5 years (n=1,550; 8.5% prevalence)	<b>adjOR= 1.8 (1.2-2.8)</b> crudeOR = 1.6 (1.1-2.4)	<u>Mother</u> : overweight <u>Child</u> : gestational age, birth weight, weight gain birth to 5 months <u>Other</u> : paternal overweight, household income
retrospective	288 children/adolescents participants of	Maternal report of smoking during	<b>smoking during pregnancy ("any"/"no") and BMI of child (<math>\chi^2</math>, 1 df)</b>		Familial risk status, prenatal

**Appendix Tables**  
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**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

Study Design	Population	Exposure Assessment	Outcome (prevalence)	Risk Estimate (95% CI)	Adjusted For			
US, Pittsburgh (Hill <i>et al.</i> 2005)	either the Cognitive and Personality Factors in Relatives of Alcoholics study or the Biological Risk Factors in Relatives of Alcoholic Women study. Participants were assessed annually and this study examines a 10 yr period (age 8-18 yr)	pregnancy assessed 0-18 years after pregnancy (most 4.9 to 10 years afterwards)	8-11 yr	$\chi^2=9.94$ (p=0.002)	exposure, gender, family ID, maternal MDD diagnosis			
			12-15 yr	$\chi^2=8.16$ (p=0.004)				
			16-18 yr	$\chi^2=4.00$ (p=0.05)				
			<b>smoking during pregnancy based on level of cigarette use and BMI of child (<math>\chi^2</math>, 2 df) "no use", "½ pack", "&gt; ½ pack"</b>					
			8-11 yr	$\chi^2=13.02$ (p=0.002)				
			12-15 yr	$\chi^2=14.04$ (p=0.001)				
16-18 yr	$\chi^2=7.22$ (p=0.03)							
prospective cohort Australia, Western (Huang <i>et al.</i> 2007)	408 children participating in the Raine Cohort recruited from King Edward Memorial hospital between 1989-1992. Children evaluated at birth, and 1, 3, 5, and 8 years of age	Maternal report of smoking during pregnancy assessed at 18 weeks pregnancy	High-risk cluster* for metabolic syndrome at age 8 (n=406; 25.1% prevalence)	OR = <b>1.82 (95%CI 1.05-3.2)</b>				
			*BMI, systolic BP, serum TG, and glucose were used as clustering variables					
prospective cohort Sweden, Nat'l registry (Iliadou <i>et al.</i> 2010)	124,203 males identified in the Swedish Medical Birth Register between 1983-1988 for whom information on BMI at ~18 years of age and maternal smoking at pregnancy was available. BMI data was obtained via military conscription records and maternal smoking data is included in the Medical Birth Register	Maternal report of smoking during pregnancy assessed at during pregnancy (usually 8-12 weeks) categorized as non-smoker, 1-9 cigarettes/day and ≥10 cigarettes/day	<b>Overweight in young adulthood (~18 years of age) BMI ≥ 25</b> crude analysis: n=124,203 and 25,295 with BMI ≥ 25(20.3%) fully adjusted: n=68,248 and 13,665 with BMI ≥ 25(20.0%)	<b>OR (95%CI)</b>	maternal age, height, BMI and pregnancy weight gain, maternal and paternal socio-economic category and education, offspring urban living, birth weight, head circumference, gestational age and age at conscription			
			0	1.00				
			1-9 cigarettes/day	adj OR = <b>1.41 (1.34-1.49)</b> crudeOR = 1.51 (1.46-1.56)				
			≥10 cigarettes/day	adjOR = 1.56 (1.46-1.66) crudeOR = 1.72 (1.65-1.80)				
			<b>Maternal smoking (yes/no) and continuous BMI</b>	<b>adjβ = 0.65 (95% CI 0.62-0.75)</b>				
<b>Maternal smoking during pregnancy</b>		<b>First son</b>	<b>Second son</b>		maternal age, height, BMI, pregnancy weight gain, maternal and paternal socio-economic category and education, and offspring birth weight, head circumference, gestational age, urban living and age at conscription			
<b>1<sup>st</sup></b>	<b>2<sup>nd</sup></b>	<b>Total n</b>	<b>Overweight n</b>	<b>adjOR (95% CI)</b>		<b>Total n</b>	<b>Overweight n</b>	<b>adjOR (95% CI)</b>
no	no	6190	979	1.00		6190	1078	1.00
yes	no	524	96	1.19 (0.87-1.63)		524	113	1.20 (0.88-1.65)
no	yes	228	46	1.15 (0.75-1.78)		228	51	0.96 (0.58-1.57)
yes	yes	1499	366	1.65 (1.35-2.01)	1499	400	1.71 (1.39-2.09)	
prospective study Germany, multi-city (Karaolis-Danckert <i>et al.</i> 2008)	370 infants from the German Multicenter Allergy study (prospective study).	Maternal report of smoking during pregnancy (assessed by questionnaire in first year after delivery)	<b>adjβ±SE of the percent rate of change between 2 and 6 years of age percent body fat</b> time x maternal smoking = 0.14 ± 0.14%/y, p = 0.3 time x maternal smoking x rapid weight gain = 0.78 ± 0.28%/y, p = 0.005	<b>% body fat:</b> Final model: as basic model and adjusted for body fat at 3 month gestational age group, firstborn, time_firstborn, time x bottle-feeding, season of birth, and time x season of birth.				
			19.3% (57/296) of kids with normal rate of growth were exposed to tobacco in utero	<b>BMI standard deviation score</b> time x maternal smoking = <b>0.06 ± 0.03, p = 0.03</b> time x maternal smoking x rapid weight gain = 0.09 ± 0.06, p = 0.10	<b>BMI:</b> Final model: as basic model and adjusted for BMI SD score at birth, gestational age group, time _ bottle-feeding.			
			28.4% (21/74) of kids with rapid rate of growth were exposed to tobacco in utero	adj OR (95% CI) for rapid weight gain between birth and 24 months 1.29 (0.66-2.49)				

**Appendix Tables**  
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**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

Study Design	Population	Exposure Assessment	Outcome (prevalence)	Risk Estimate (95% CI)	Adjusted For
prospective cohort UK (Leary <i>et al.</i> 2006)	Analysis was based on 5689 white singletons born in 1991–1992 and enrolled in the Avon Longitudinal Study of Parents and Children	Maternal report of smoking during pregnancy (28-32 weeks of gestation) based on questionnaire at 32 weeks gestation; 19.8% reported smoking in a least one trimester	<b>regression of offspring BMI at mean age 9.9 years</b>		sex and child's age at DXA scan; maternal, partner, social, and infant feeding factors; birth weight and gestation
			smoking during any trimester (n=3,621)	<b>0.24 (0.16-0.32), p&lt;0.001</b>	
			smoking during 1 <sup>st</sup> trimester (n=3,621)	0.25 (0.16-0.33), p<0.001	
			smoking during 2 <sup>nd</sup> trimester (n=3,621)	0.24 (0.15-0.34), p<0.001	
			smoking during 3 <sup>rd</sup> trimester (n=3,621)	0.28 (0.18-0.37), p<0.001	
			<b>smoking status of partner analysis</b>		
			maternal smoking (n=3,606)	0.24 (0.16-0.32), p<0.001	
partner smoking (3,606)	0.11 (0.05-0.18), p=0.001				
prospective cohort Japan, Enzan City (Mizutani <i>et al.</i> 2007)	1417 mother-child pairs enrolled in Project Enzan between 1991 and 1999. Children were assessed at 5 years of age.	Maternal report of current smoking assessed during early pregnancy (most before week 16 of pregnancy)  *overweight and obesity criteria not explicit, but assumed to be based on standard criteria used in Europe.	<b>current smoking during early pregnancy versus "had quit" or "never smoked"</b>		Breast feeding, education, smoking, sleep duration time, and breakfast adjusted for maternal age and maternal BMI
			Overweight at 5 years: BMI ≥90 <sup>th</sup> to < 97 <sup>th</sup> percentile* (n=1,417; 11% prevalence)	<b>adjOR = 2.15 (1.12-4.11)</b> crudeOR = 2.29 (1.28-4.08)	
			Obese at 5 years: BMI ≥ 97 <sup>th</sup> percentile* (n=1,417; 2.7% prevalence)	<b>adjOR = 3.93 (1.46-10.56)</b> crudeOR = 5.14 (2.27-11.64)	
prospective cohort Great Britain (Montgomery and Ekbohm 2002)	Diabetes and obesity in the offspring of women in the British National Child Development Study (Perinatal Mortality Survey) from March 3-9, 1958 (4,945 total women and their offspring in 1974)	Maternal report of smoking while pregnant, after 4 <sup>th</sup> month (30.2% reported smoking)	Diabetes in child at age 16 (n=4,945; 28 with diabetes (0.56%)/4,917 without diabetes		Maternal smoking during pregnancy, maternal smoking in 1974, sex, mother's age at birth, age mother left school, social class at birth, birth weight, smoking at age 16 years, and BMI at 33 years
			Non-smoker	OR= 1.00	
			Medium-smoker	adjOR = 1.01 (0.23-4.53) crudeOR = 1.24 (0.35-4.42)	
			Varies between medium and heavy	adjOR = 3.55 (0.88 – 14.38) crudeOR = 4.13 (1.32-12.88)	
			Heavy smoker [n=530, 9 cases (1.72%)]	<b>adjOR = 4.02 (1.14 – 14.14)</b> crudeOR = 4.94 (2.07-11.77)	
			Obesity in child at age 33 (BMI > 30), with diabetics excluded (10%, or n=602, total n not reported)		
			Non-smoker	OR= 1.00	
Medium-smoker	1.34 (1.07-1.69)				
Varies between medium and heavy	1.35 (0.95-1.92)				
Heavy smoker	<b>adjOR = 1.38 (1.06-1.79)</b>				
prospective cohort US, Massachusetts (Oken <i>et al.</i> 2005)	746 mother-child pairs from Project Viva (Massachusetts), singleton pregnancy enrolled at < 22 weeks gestation; 50% boys; children assessed at 3 years of age.	Maternal report of smoking during early pregnancy (10%)	overweight at 3 years, BMI ≥85 <sup>th</sup> percentile [n=754; 204 overweight (27%)]	<b>adjOR = 2.2 (1.2 -3.9)</b> crudeOR= 2.5 (1.5-4.1) <sup>1</sup>	Mother: pre-pregnancy BMI, gestational weight gain, parity, race/ethnicity, education, income, age, fetal growth, gestation length, 3 <sup>rd</sup> trimester SBP. Father: BMI Child: sex, age
			subscapular + triceps skinfold thickness (mm)	adjOR = 2.0 (0.9 -3.0) crudeOR= 2.2 (1.1-3.2)	
			higher BMI z-score	0.30 units (0.05-0.55)	
prospective cohort UK (Power <i>et al.</i> 2010)	8,815 men and women in a 1958 British cohort	Maternal smoking recorded at birth, prospective study (32.2% reported smoking after 4 <sup>th</sup> month of pregnancy)	<b>Obesity at 45 years of age (BMI ≥ 30)</b>	<b>adj OR = 1.40 (1.25 to 1.56)</b> crudeOR = 1.38 (1.25-1.54)	Social class, education, physical activity and inactivity (TV/PC use), smoking and consumption of fruit/vegetables, cakes/sweets and alcohol
			<b>Metabolic syndrome at 45 years</b>	adj OR = 0.55 (0.47 to 0.64) crudeOR = 1.21 (1.05-1.39)	

**Appendix Tables**  
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**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

Study Design	Population	Exposure Assessment	Outcome (prevalence)	Risk Estimate (95% CI)	Adjusted For
prospective cohort UK (Power and Jefferis 2002)	16,766 singleton births in England, Wales and Scotland on 3-9 March, 1958.; 52% male Children assessed at 7, 11, 16, 23, and 33 years of age. BMI data available for 5,839 at age 33 (2,918 ♂; 2,921 ♀)	Maternal report at birth of smoking  5,624 (33.5%) reported smoking after the 4 <sup>th</sup> month of pregnancy	<b>Obesity at 33 years of age (BMI ≥ 30)</b> ♂ (n=2,918; prevalence not reported)  ♀ (n=2,921; prevalence not reported)	<b>adjOR = 1.55 (1.19-2.00)</b> crudeOR = 1.56 (1.22-2.00)  <b>adjOR = 1.45 (1.13-1.87)</b> crudeOR = 1.41 (1.12-1.79)	<u>Mother</u> : BMI in 1969 <u>Child</u> : infant feeding; birth weight; social class at birth, 7 years, & 33 years; diet at 33 years; physical inactivity at 23 years
prospective cohort UK (Reilly <i>et al.</i> 2005)	8234 children born between April 1991 and December 1992 participating in the Avon Longitudinal Study [51% boys, 6.9 to 8.5 years old (median age=7.6)]  *5,493 children had complete data for multivariable analysis	Maternal report of smoking during pregnancy (28-32 weeks of gestation) based on questionnaire at 32 weeks gestation; 14% reported smoking	<b>Obesity at age 7, BMI ≥95<sup>th</sup> percentile [n=7758; 671 obese (8.6%)]</b> none [n=5,889; 443 obese (7.5%)] 1-9 cigarettes/day [n=434; 50 obese (11.5%)] 10-19 cigarettes/day [n=423; 48 obese (11.3%)] ≥ 20 cigarettes/day [n = 138; 19 obese (13.8%)]	OR=1.00 <b>adjOR = 1.76 (1.21-2.52)</b> crudeOR=1.60 (1.17-2.18) adjOR=1.59 (1.08-2.34) crudeOR=1.57 (1.15-2.16) adjOR=1.80 (1.01-3.99) crudeOR=1.96 (1.20-3.22)	birth weight, sex, parity, season of birth, gestational age, number of fetuses, infant feeding, parental obesity, number of siblings, ethnicity, mother's age, television, time in car, night-time sleep, dietary patterns
prospective cohort USA (Salsberry and Reagan 2005)	3,022 children of original enrollees in the National Longitudinal Survey of Youth's Child-Mother study(born 1978-1964); the study children were born between 1982 and 1996; 51% boys; Evaluated at 2-3 years old (mean=35 months) 4-5 years old (mean=60 months); 6-7 years old (mean=84 months)	Maternal report smoking during pregnancy, yes/no (not reported when assessed); 29% reported smoking	<b>Overweight, BMI ≥95<sup>th</sup> percentile</b> 2-3 years old [n=3022; 580 overweight (19%)] 4-5 years old [n=3022; 406 overweight (13%)] 6-7 years old: [n=3022; 372 overweight (12%)]	OR=1.37 (1.08-1.73) OR=1.43 (1.11-1.84) <b>adjOR=1.74 (1.32-2.29)</b> crudeOR=1.43	Maternal age at time of birth, race/ethnicity, breastfeeding, birth weight for gestational age, gender, parity, maternal pre-pregnancy BMI, year of birth, education, marital status. Child's age, weight
cross-sectional Canada Syme <i>et al.</i> (2010)	508 total adolescents, 12-18 years of age, recruited from high schools from a French-Canadian founder population in Quebec. Subjects matched at recruitment by maternal education and subject's school. Data collected on puberty (using puberty development scale), body weight and MRI for total, subcutaneous, and intra-abdominal fat n assessed during early puberty (stages 1-3) = 163 n assessed during late puberty (stages 4-5) = 341	Maternal report smoking during pregnancy  237 exposed to prenatal maternal smoking (E)/268 not exposed (NE)  early puberty: n=163; 92 (E) and 71 (E) late puberty: n=341; 165 (E) and 176 (E)	<b>adolescents exposed (E) or non-exposed (NE) to maternal smoking</b> body weight (kg), early puberty ~50 (NE) vs ~49 (E); p=NS body weight (kg), late puberty ~60 (NE) vs ~64 (E); p=0.02 total body fat (kg), early puberty ~10 (NE) vs ~9 (E); p=NS total body fat (kg), late puberty ~12 (NE) vs ~15 (E); p=0.003 subcutaneous fat (mm <sup>3</sup> ), early puberty ~105,000 (NE) vs ~92,000 (E); p=NS subcutaneous fat (mm <sup>3</sup> ), late puberty ~105,000 (NE) vs ~ 130,000 (E); p = 0.004 intra-abdominal fat (mm <sup>3</sup> ), early puberty ~25,000 (NE) vs ~ 21,000 (E); p=NS intra-abdominal fat (mm <sup>3</sup> ), late puberty ~22,000 (NE) vs ~ 30,000 mm <sup>2</sup> ; p = 0.001	Group differences were analyzed with mixed-model analysis with puberty (early vs. late) and prenatal exposure to maternal cigarette smoking (PEMCS) (E vs. NE) as main factors, while adjusting for sex differences. Log-transformed values of subcutaneous and intra-abdominal fat were analyzed.	
prospective cohort UK Thomas <i>et al.</i> (2007)	7,518 participants of the 1958 British Birth Cohort with information on A1C assessed at 45 years of age	Maternal report of smoking during pregnancy (31% reported some degree of smoking)	<b>Glucose metabolism (A1C ≥ 6%) at 45 years of age</b> adjOR (95%CI), basic model = 1.33 (1.04-1.71) adjOR (95%CI), basic model + birth weight for gestational age + adult adiposity = <b>1.01 (0.78-1.32)</b>	gestational age, preeclampsia, maternal pre-pregnancy BMI, socioeconomic status at birth, family history of diabetes, sex	
prospective cohort Brazil (Tome <i>et al.</i> 2007)	2,797 children recruited to a study in 1978/79 as infants from 8 hospitals in Ribeirao and assessed as 8-10 years of age in 1987/89 (50.6% male)	Maternal report smoking during pregnancy, assessed at birth (25.4% reported smoking)	<b>overweight at 8-10 years</b> (6.4% were obese, BMI≥95 <sup>th</sup> percentile; 9.3% were overweight, BMI 85 to 94.9 <sup>th</sup> 9.5% were malnourished, BMI<5 <sup>th</sup> percentile) BMI ≥85 <sup>th</sup> versus 5 to 84.9 percentile 378 with BMI ≥85 <sup>th</sup> (13.5%) BMI 5 to 84.9 <sup>th</sup> versus <5 <sup>th</sup> percentile	<b>adjOR = 1.07 (0.84-1.37)</b> crudeOR= 0.93 (0.74-1.19) adjOR = 0.56 (0.40-0.78)	newborn sex, number of pregnancies, preterm birth, birth weight, mother's marital status, maternal schooling, type of school

**Appendix Tables**  
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**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

Study Design	Population	Exposure Assessment	Outcome (prevalence)	Risk Estimate (95% CI)	Adjusted For	
				crudeOR=0.65 (0.47-0.90)		
			*author's concluded that maternal smoking was protective against malnutrition (based on having BMI <5 <sup>th</sup> percentile)			
cross-sectional Bavaria, Germany (Toschke <i>et al.</i> 2002)	8,765 German school children in Bavaria, taking part in the 1997 school entrance health examination, whose parents completed a questionnaire on risk factors for atopic diseases (52% boys, 5 – 6.9 years of age) (total cohort of 8,765 but precise period of smoking could not be identified in 400 mothers, so 8,365 used in analysis)	Maternal report smoking before, during and after pregnancy (7.5% smoked throughout pregnancy)	<b>Overweight at 5-6.9 years, BMI ≥90<sup>th</sup> percentile (10% prevalence<sup>1</sup>)</b>		Breast feeding, parental education low birth weight, prematurity	
			never smoked (n=5,919; 9.1% overweight)	OR=1.00		
			smoked before or before and after (but not during) pregnancy (n=1,542; 14.1% overweight)	OR=1.63 (1.37-1.94)		
			smoked throughout pregnancy (n=660; 15.6% overweight)	<b>adjOR=1.58 (1.23-2.04)</b> crudeOR=1.85 (1.47-2.33)		
			smoked only after pregnancy (n=244; 7.4% overweight)	0.80 (0.48-1.32)		
			<b>Obese at 5-6.9 years, BMI ≥97<sup>th</sup> percentile (3% prevalence<sup>1</sup>)</b>			
			never smoked (n=5,919; 2.8% obese)	OR=1.00		
			Smoked before or before and after (but not during) pregnancy (n=1,542; 4.5% obese)	1.74 (1.29-2.34)		
			Smoked throughout pregnancy (n=660; 6.2% obese)	<b>adjOR= 1.92(1.29-2.86)</b> crudeOR=2.32(1.63-3.3)		
			smoked only after pregnancy (n=244; 1.6% obese)	0.63 (0.23-1.73)		
cross-sectional Bavaria, Germany (Toschke <i>et al.</i> 2003)	4,974 German school children in Bavaria, taking part in the 2001-2002 school entrance health examination, and whose parents completed the questionnaire; (53% boys, 5 – 6.9 years of age). Data from 4,706 children used in analysis	Maternal report smoking before, during and after pregnancy (10.9% smoked during early pregnancy)	<b>Overweight at 5-6.9 years of age, ~BMI ≥ 25 (n=4,706; 10.4% prevalence<sup>1</sup>)</b>		breastfeeding, parental educational level, parental obesity, child television watching, playing electronic games, physical activity, high infant weight gain.	
			Smoking during early pregnancy	<b>adjOR=1.52 (1.14-2.01)</b> crudeOR=1.66 (1.27-2.18)		
			Smoking throughout pregnancy	adjOR=1.23 (0.89-1.70) crudeOR=1.85 (1.38-2.47)		
			<b>Obese at 5-6.9 years of age, ~BMI &gt;30 [n=4,706; 2.7% prevalence<sup>1</sup>)</b>			
			Smoking during early pregnancy	<b>adjOR=2.22 (1.33-3.69)</b> crudeOR=2.41 (1.49-3.91)		
			Smoking throughout pregnancy	adjOR=1.70 (1.02-2.87) crudeOR=3.23 (2.00-5.21)		
cross-sectional Bavaria, Germany (von Kries <i>et al.</i> 2002)	6,483 German school children in Bavaria, taking part in the 1999 school entrance health examination, and whose parents completed the questionnaire; [% boys not stated]; Age range= 5.00 – 6.99 years	Maternal report smoking during pregnancy (9.8% reported smoking during pregnancy)	<b>Overweight at 5-6.9 years, BMI ≥90<sup>th</sup> percentile (10% prevalence<sup>1</sup>)</b>	<b>adjOR=1.43 (1.07-1.90)</b> crudeOR=1.97 (1.52-2.56)	Parental education and BMI, birth weight, breast feeding, watching television/playing video games, and snacking in front of the television	
			<b>Obese at 5-6.9 years, BMI ≥97<sup>th</sup> percentile (3% prevalence<sup>1</sup>)</b>	<b>adjOR=2.06 (1.31-3.23)</b> crudeOR=2.96 (1.97-4.46)		
			<b>Prevalence based on cigarettes/day</b>	<b>obese</b>		<b>overweight</b>
			0	8.1		2.2
			<10/day	14.1		5.7
≥10/day	17	8.5				

**Appendix Tables**  
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**Appendix Table A. Epidemiology studies of smoking during pregnancy and overweight/obese/diabetes in offspring**

Study Design	Population	Exposure Assessment	Outcome (prevalence)		Risk Estimate (95% CI)		Adjusted For
			p-trend		p<0.001	p<0.001	
cross-sectional Bavaria, Germany (von Kries <i>et al.</i> 2008)	5,899 German school children in Bavaria, taking part in the 2005 school entrance health examination, and whose parents completed the questionnaire; 52% Boys; Mean age = 5.8 years	Maternal and paternal report smoking, at time of interview and maternal before or during pregnancy (20.9% reported smoking before or during pregnancy)	<b>Overweight at 5-6.9 years of age, BMI ≥ 25 (n=5,899; 13.7% overweight)</b>				Maternal/paternal BMI and education, child's birth weight, premature birth, breastfeeding, child's physical activity and TV watching, paternal smoking status at interview
			Mother currently smoking		OR=1.3 (1.0-1.7)		
			Father currently smoking		1.4 (1.1-1.7)		
			Mother smoked before or in pregnancy		<b>adjOR=1.3 (1.1-1.7)</b>	crudeOR=1.7(1.4-1.9)	
			<b>Obese at 5-6.9 years of age, BMI &gt;30 (n=5,899; 3.9% obese)</b>				
			Mother currently smoking		2.5 (1.7-3.7)		
			Father currently smoking		1.3 (0.9-1.9)		
		Mother smoked before or in pregnancy		<b>adjOR=1.9 (1.3-2.7)</b>	crudeOR=2.3(1.8-3.1)		
retrospective cohort US, Ohio (Whitaker 2004)	8,494 low-income children, singleton births in 1992-1996, who were enrolled in the Ohio WIC program, and followed from the first trimester of pregnancy through 24 to 59 months of age (50% boys; children evaluated at 2 years, 3 years and 4 years of age)	Maternal report smoking during pregnancy (32.9% reported smoking)	<b>Obesity, BMI ≥ 95<sup>th</sup> percentile</b>				maternal BMI, age, parity, pregnancy weight gain, education, marital status, age, race/ethnicity, fetal growth, sex, year of birth
			Age 2 [n=6,764; 643 obese (9.5%)]		adjOR=1.43 (1.19-1.72)	crudeOR=1.20 (1.02-1.42) <sup>1</sup>	
			Age 3 [n=6,063; 758 obese (12.5%)]		adjOR=1.25 (1.05-1.49)	crudeOR=1.09 (0.93-1.28) <sup>1</sup>	
			Age 4 [n=5,089; 753 obese (14.8%)]		<b>adjOR=1.21 (1.01-1.45)</b>	crudeOR=1.06 (0.90-1.24) <sup>1</sup>	
prospective cohort Norway (Trondheim) and Sweden (Uppsala)  (Widerøe <i>et al.</i> 2003)	A random sample of 482 women selected from a population cohort of 5,772 followed during pregnancy until the child was 5 years old; (49% boys, children assessed at 5 years of age)	Maternal report smoking during pregnancy, assessed at 17 weeks of gestation (yes/no: 31.3% yes)	Overweight at 5 years, BMI≥85 <sup>th</sup> percentile [n=336; 50 overweight (14.9%)]			<b>adjRR=3.8 (2.0-7.2)</b>	Maternal diet, breast feeding, maternal obesity, socio-economic status, birth weight
			Overweight, sum of skin fold thickness (SFT)* ≥85 <sup>th</sup> percentile [n=315; 48 overweight (15.2%)/267 not overweight]			crudeRR=3.0 (1.6-5.6)	
			*subscapular + triceps skinfold thickness (mm)			adjRR=2.2 (1.1-4.1)	
			Overweight at 5 years based on number of cigarettes smoked/day			crudeRR=2.0 (1.1-3.8)	
			BMI, 1-10 cigarettes/day		2.3 (1.3-4.0)		
			BMI, >10 cigarettes/day		3.1 (1.8-5.3)		
			SFT, 1-10 cigarettes/day		1.8 (1.0-3.1)		
SFT, >10 cigarettes/day		2.2 (1.2-3.9)					

<sup>1</sup>Reported in Oken *et al.* (2008)

Data in blue are presented in the forest plot (Figure 1)

**Appendix Tables**  
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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

<b>Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine</b>			
Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Bruin <i>et al.</i> 2007)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day on one of the following schedules:</p> <ul style="list-style-type: none"> <li>• 2 weeks prior to mating (A)</li> <li>• 2 weeks prior to mating until parturition (B)</li> <li>• 2 weeks prior to mating until weaning (C)</li> <li>• 2 weeks prior to mating and then parturition until weaning (D)</li> </ul> <p>F1 males: OGTT was conducted at 26 weeks; pancreatic <math>\beta</math>-cell mass (% area), % <math>\beta</math>-cell apoptosis, and % islet cell proliferation (TUNEL) were assessed at 4 and 26 weeks.</p>	5 dams/ 12 F1 males per group	1A, sc injection	No effects
		1B	<p>↓ Pancreatic <math>\beta</math>-cell mass on PND1 (~68% of control) and at 4 weeks (~71% of control)</p> <p>↑ Percentage of PCNA<sup>+</sup> islet cells at 4 weeks (~1.34-fold)</p>
		1C	<p>↑ Total glucose (AUC) [data not shown]</p> <p>↑ Peak glucose concentration at 30 min (1.19-fold)</p> <p>↑ Serum glucose level at 120 min (1.39-fold)</p> <p>↑ Total Insulin (AUC) (1.54-fold)</p> <p>↓ Pancreatic <math>\beta</math>-cell mass for fetuses and at 4 weeks (~76% of control) and 26 weeks (~60% of control)</p> <p>↑ % <math>\beta</math>-cell apoptosis (2.38-fold)</p>
		1D	<p>No effects</p> <p><b>No effect findings in any group:</b> Maternal body weight, food consumption, mating success, litter size, pup birth weight.</p>
<p><b>(Bruin <i>et al.</i> 2008a)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection (saline) at 0 or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. F1 males: On PND21, pancreatic tissue was collected for Western Blot analysis, immunohistochemistry and electron microscopy.</p>	10 dams/ 5 F1 males per group	1, sc injection	<p><u>Mitochondrial-Mediated Apoptosis:</u></p> <p>↓ Bcl2 expression; ↓ Ratio of Bax expression in the cytosolic fraction relative to the mitochondrial fraction</p> <p>↑ Ratio of cytochrome c expression in the cytosolic fraction relative to the mitochondrial fraction</p> <p>↑ 17-kDa active form of caspase-3</p> <p>↑ Ratio of active to inactive caspase-3 protein (3.3-fold)</p> <p><u>Electron Microscopy:</u></p> <p>↑ Average optical intensity of the mitochondria (1.11-fold)</p> <p><b>No effect findings:</b> Death receptor-mediated apoptosis, immunohistochemical localization of active Caspase-3, number of mitochondria per <math>\beta</math>-cell, average mitochondrion area within the <math>\beta</math>-cells.</p>

**Appendix Tables**  
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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Bruin <i>et al.</i> 2008b)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. F1 males: Pancreatic tissue was collected on PND1 and PND21 for RT (n=6/group) and real-time PCR (n=4/group), and on PND21 for Western Blot analysis (n=4/group), protein carbonyl detection in homogenates (n=5/group), and protein carbonyl detection in the mitochondrial fraction (n=4/group).</p>	<p>10 dams/ 4 – 6 F1 males per group</p>	<p>1, sc injection</p>	<p>↑Protein expression of GPx-1 and MnSOD (but no effect on HO-1 or CuZnSOD) ↑Oxidative damage to a 25-kDa protein ↑Total protein carbonyl levels in the mitochondrial fraction (~3-fold) ↑ Reactive oxygen species (ROS)</p> <p><b>No effect findings:</b> real-time PCR mRNA expression of antioxidant proteins, nAChR subunit mRNA expression, 8-iso prostaglandin F<sub>2α</sub></p>
<p><b>(Bruin <i>et al.</i> 2008c)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. F1 males: at 3-4, 15 and 26 weeks, an OGTT was performed (n=15/group) and pancreatic tissue was collected for electron microscopy (n=4/group/ time point) and enzyme activity assays (n=5-7/ group/ time point).</p>	<p>30 dams/ 25- 26 F1 males per group</p>	<p>1 mg/kg bw/day</p>	<p>↑Total glucose (AUC) at 15 weeks (~1.11-fold) and 26 weeks (~1.08-fold) ↑Individual mitochondrial area at 15 weeks (~1.45-fold) and 26 weeks (~2.74-fold) ↑Proportion of mitochondria with blebbing or merging with a neighbor at 15 weeks (~4.0-fold) and 26 weeks (~11.0-fold) ↓Proportion of stage 1 mitochondria (healthy) at 3 weeks (~74% of control), 15 weeks (~40% of control) and 26 weeks (~12% of control) ↑Proportion of stage 2 mitochondria at 3 weeks (~4.7), increased at 15 weeks (~2.0) and 26 weeks (~1.8) Increased* proportion of stage 3, 4 or 5 mitochondria at 15 weeks (~200-fold) and 26 weeks (~570-fold) ↓Mitochondrial Complex IV enzyme activity (~81% of control) by week 26 ↓Total insulin granules (68% of control) by week 26 ↓Number of immature granules per β-cell (14% of control) by week 26 ↑ Islet ROS production (~1.2- fold) at week 26 ↑ Islet protein carbonyls (~1.35- fold) at week 26 ↓Glucose-stimulated insulin secretion at week 26</p> <p><b>No effect findings:</b> OGTT at 4 weeks; citrate synthase activity (an indicator of mitochondrial mass) at 26 weeks.</p>

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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Oliveira <i>et al.</i> 2010a)</b> Female Wistar rats received nicotine by subcutaneous osmotic mini pump infusion at 0 (saline) or 6 mg/kg bw/day from PND3 – PND16. Mean cotinine levels on PND15 were 225.8 ng/mL in milk, 239 ng/mL in maternal serum, and 20.4 ng/mL in pup serum. F1 males were killed on PND180; blood was collected. Western Blot assay was conducted on the hypothalamus; liver and muscle glycogen were determined; adipose distribution was evaluated, morphometric analysis of visceral and epididymal fat was conducted, and Lee's index of obesity was calculated.</p>	<p>6 dams/ 36 F1 males per group</p>	<p>6, sc mini-pump</p>	<p>↑Body weight gain PND75 – PND100 (1.10-fold) and after PND165 (1.10-fold)            ↑Lee's index of obesity (1.07-fold)            ↑Central (1.10-fold) and total (1.12-fold) adiposity            ↑Size of adiposities (epididymal – 1.12-fold; inguinal – 1.43-fold)            ↑Serum leptin (2.13-fold)            ↓Serum Apo Ai protein (36% of control)            ↓Liver glycogen (92% of control)            ↑Muscle glycogen (2.20-fold)            ↓Adiponectin:fat mass ratio (76% of control)            ↑Leptin:adiponectin ratio (LAR) (1.98-fold)            ↑Blood insulin (1.56-fold)            ↑Insulin resistance index (IRI) (1.43-fold)            ↓Hypothalamic OB-R (39% of control), JAK2 (59% of control), p-STAT3 (44% of control)            ↓Ratio of hypothalamic p-STAT3 to total p-STAT (60% of control)            ↑Hypothalamic SOCS3 expression (1.81-fold)</p> <p><b>No effect findings:</b> Body length; food intake; subcutaneous fat; serum cholesterols, globulins, albumin and total protein; fasting blood glucose, corticosterone, or adiponectin.</p>
<p><b>(Gao <i>et al.</i> 2005)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. F1 males: At 26 weeks, fat pads were weighed and the morphology of the aorta and mesenteric arteries was studied (n=4 or 5/group).</p> <p><u>Also presented in study, but not summarized here:</u> The effect of perivascular adipose tissue (PVAT) on vessel (aorta) reactivity <i>in vitro</i>.</p>	<p>15-16 F1 males/group</p>	<p>1, sc injection</p>	<p>↑Body weight at 10 weeks (~1.04-fold), 15 weeks (~1.10-fold), 20 weeks (~1.11-fold) and 26 weeks (1.13-fold)            ↑Fat pad weight – epididymal (1.48-fold), mesentery (1.32-fold), perineal (1.45-fold), and total (1.42-fold)            ↑Cross-sectional area of the perivascular adipose tissue (PVAT) – thoracic aorta (1.46-fold) and mesenteric artery (1.47-fold)</p> <p><b>No effect findings:</b> Weight of the left ventricle.</p>

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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Gao <i>et al.</i> 2008)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. F1 males: Blood pressure was measured starting at 14 weeks; at 26 weeks, aorta and mesenteric arteries were collected.</p> <p><u>Also presented in study, but not summarized here:</u> Structural characteristics of the kidneys; immunohistochemical staining of perivascular fat; contractile response of mesenteric arteries <i>in vitro</i></p>	6/group	1, sc injection	<p>↑Systolic blood pressure at 14 weeks (1.08-fold), 18 weeks (1.09-fold) and 24 weeks (1.11-fold)</p> <p><b>No effect findings:</b> Body weight, total of white fat pad weights, interscapular brown fat weight, perivascular adipose tissue, arterial area of the aorta or mesenteric arteries.</p>
<p><b>(Grove <i>et al.</i> 2001)</b> Pregnant rhesus monkeys received nicotine tartrate by subcutaneous osmotic mini pump infusion at 0 (saline) or 1.5 mg/kg bw/day from GD26 – GD160. The mean nicotine level in amniotic fluid on GD160 was 13.8 ng/mL. Fetuses were delivered by C-section on GD160; brain, hypothalamus, pancreas and adrenals were collected. <i>In situ</i> hybridization of hypothalamus was conducted.</p>	6-7/group	1.5, sc mini-pump	<p>↓Fetal pancreas weight (65% of control) ↓Amniotic fluid cortisol levels on GD118 (~64% of control) and GD160 (~76% of control) – but not GD130 ↓Fetal serum leptin levels (~50% of control) ↓NPY mRNA expression (~56% of control) in the arcuate nucleus of the hypothalamus Increased POMC mRNA expression (2-fold) in the arcuate nucleus of the hypothalamus</p> <p><b>No effect findings:</b> Fetal body weight and crown-rump length; maternal serum and amniotic fluid leptin levels.</p>
<p><b>(Gruslin <i>et al.</i> 2009)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. Fetal blood was collected from 5 litters/group on GD15, 18 and 21. Five dams/group were allowed to deliver their litters; pup blood was collected on PND1 and PND21. IGF-II profiles were determined by Sensitive Western Blot analysis.</p>	5 dams/ 20 offspring per group	1, sc injection	<p>↓Fetal body weight on GD21 (92% of control) and pup weight on PND1 (86% of control) "Nicotine exposure prevented the decrease in maternal IGF-II processing seen in controls with advancing gestation."</p> <p><b>No effect findings:</b> Maternal serum levels of big IGF-II (1-87); fetal IGF-II profile.</p>
<p><b>(Huang and Winzer-Serhan 2007)</b></p>	17-41 for	0.5, oral	<p>↓ body weight (~93% of control)</p>

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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
Sprague-Dawley rats with 0 (Enfamil® milk formula), 0.25, 1.5, or 3 mg/kg nicotine twice a day for seven days (0, 0.5, 3, and 6 mg/kg-d) and once on PND8 by oral gavage. Litters were adjusted to 9-12 pup on PND1. Pups were assessed on PND8 for serum leptin and brains were frozen and analyzed by <i>in situ</i> hybridization for feeding proteins and receptor autoradiography for receptor binding studies	body weight; 5-16 for leptin; 3/group mRNA		↑ nicotinic receptor binding in Arc and VMN assessed by <sup>125</sup> I epibatidine binding for heteromeric α4β2* receptors (no differences in <sup>125</sup> I-α-BTX binding for homomeric α7 receptors) ↑ NPY mRNA in Arc (1.27-fold) ↑ AgRP mRNA in Arch (1.53-fold) ↑ POMC mRNA in Arch (1.3-fold)
		3	↓ body weight (~87% of control) ↑ serum leptin (1.66-fold) ↑ nicotinic receptor binding in Arc and VMN assessed by <sup>125</sup> I epibatidine binding (no differences in <sup>125</sup> I-α-BTX binding) ↑ NPY mRNA in Arc (1.42-fold) ↑ AgRP mRNA in Arch (1.59-fold) ↑ POMC mRNA in Arch (1.46-fold)
		6	↓ body weight (~80% of control) ↑ serum leptin (1.9-fold) ↑ nicotinic receptor binding in Arc and VMN assessed by <sup>125</sup> I epibatidine binding (no differences in <sup>125</sup> I-α-BTX binding) ↑ NPY mRNA in Arc (1.28-fold) ↑ AgRP mRNA in Arch (1.56-fold) ↑ POMC mRNA in Arch (1.39-fold)
			mRNA expression effects were blocked by dihydro-b-erythroidine (DHβE), an α4β2* nAChR antagonist; effect on leptin only partially blocked by DHβE <b>No effect findings:</b> CART mRNA in Arc

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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Holloway <i>et al.</i> 2005)</b> Female Wistar rats received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. Apoptotic pancreatic <math>\beta</math>-cells were detected on PND1 by TUNEL assay. F1 males: OGTT were conducted at 7 and 26 weeks.</p>	<p>4 dams/ 16 F1 males per group</p>	<p>1, sc injection</p>	<p>↓Birth weight (96% of control)            ↑Total growth response (AUC) at 26 weeks (1.16-fold)            ↓Serum insulin on PND1 (64% of control)            ↑Islet apoptosis on PND1 (2.07-fold)            ↑TUNEL/insulin-positive <math>\beta</math>-cells (3.16-fold)  <u>OGTT at 7 weeks</u>            ↑Serum glucose at 120-minutes (~1.58-fold)            ↑Serum triglyceride (1.33-fold)   <u>OGTT at 26 weeks</u>            ↑Serum glucose at 30 minutes (~1.41-fold)            ↑Serum insulin at baseline (~2.86-fold), 30 minutes (~4.55-fold), and 120 minutes (~2.79-fold)            ↑Serum triglyceride (1.18-fold)   <b>No effect findings:</b> Litter size, sex ratio, gestational length; weight gain during lactation; <math>\beta</math>-cell area on PND1.</p>
<p><b>(Holloway <i>et al.</i> 2007)</b> Female Wistar rats (F0 generation) received nicotine bitartrate by subcutaneous injection at 0 (saline) or 1 mg/kg bw/day from 2 weeks prior to mating until weaning. F1 females (not treated) were used to produce the F2 generation (also not treated). At 13 and 15 weeks, the F2 generation was evaluated for glucose homeostasis (IPGTT), serum lipids, fat pad weights, blood pressure and mitochondrial enzyme activity in muscle.   <u>Also presented in study, but not summarized here:</u>            F2 fertility or reproductive parameters;</p>	<p>not reported</p>	<p>1, sc injection</p>	<p>↓F1 body weight at weaning (94% of control)            Increased % body fat at 15 weeks (1.18-fold)            ↑Serum leptin at 15 weeks (1.33-fold)            ↑Serum insulin at baseline (2.25-fold)            ↑Serum cholesterol at 15 weeks (1.10-fold)   <u>Blood Pressure at 15 weeks</u>            ↑Systolic BP (1.17-fold)            ↑Diastolic BP (1.29-fold)            ↑Mean arterial pressure (MAP) (1.18-fold)   <b>No effect findings:</b> F2 birth weight; serum glucose during IPGTT at 15 weeks; serum triglycerides at 15 weeks; non-esterified free fatty acids at 15 weeks; mitochondrial enzyme activity.</p>
<p><b>(Jose <i>et al.</i> 2009)</b> Male Wistar rats (30 days old) received nicotine as 5 daily subcutaneous injections at 0 (saline) or 5</p>	<p>6/group</p>	<p>5, sc injection for 28 days</p>	<p>↓Hepatic glycogen (60% of control)</p>

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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
mg/kg bw/day for 28 or 56 days. Mean plasma cotinine levels were 633.6 ng/mL after 28 days and 638.1 ng/mL after 56 days.		5, sc injection for 56 days	↓Body weight (86% of control) ↓Liver weight (80% of control) ↓Hepatic glycogen (54% of control)
<u>Also presented in study, but not summarized here:</u> <i>In vitro</i> experiments on PBG-synthase and AChE activity in blood and brain.			<b>No effect findings:</b> Brain weights; plasma glucose; <i>in vivo</i> blood and brain PBG-synthase activity; <i>in vivo</i> blood and brain AChE activity
<b>(Newman et al. 1999)</b> Pregnant Sprague-Dawley rats received nicotine by subcutaneous osmotic mini pump infusion at 0 (untreated or saline), 0.75, 1.5, or 3 mg/kg bw/day for 35 days starting GD 3 [through PND16]; one group remained untreated.	4 dams/ 10-15 pups per group	0.75, sc mini-pump	<u>Pup body weight (litter weight)</u> ↓At birth (78% of control) ↑PND14 (1.43-fold) ↑PND21 (1.30-fold)
		1.5	<u>Pup body weight (litter weight)</u> ↓At birth (88% of control) ↑PND14 (1.55-fold) ↑PND21 (1.49-fold)
		3	<u>Pup body weight (litter weight)</u> ↓At birth (72% of control) ↑PND14 (1.43-fold) ↑PND21 (1.37-fold)
<u>Also presented in study, but not summarized here:</u> F0 fertility or reproductive parameters; hyperactivity in F1 generation.			<b>No effect findings:</b> Dam body weight at implantation and parturition.
<b>(Oliveira et al. 2009)</b> Wistar rat dams received nicotine by subcutaneous osmotic mini pump infusion at 0 (saline) or 6 mg/kg bw/day from PND2 through PND15. Two pups/litter were killed on PND15, PND21 and PND180, or (in a second experiment) PND15, PND90 and PND180; blood, visceral fat masses (VFM), and carcasses were collected.	12 dams/ 72 pups per group	6, sc mini-pump	↑Body weight from PND75 – PND100 (1.10-fold) and PND165 – PND180 (1.10-fold) ↑Total fat mass on PND15 (1.27-fold), PND90 (1.25-fold) and PND180 (1.33-fold). ↑VFM at all ages (~1.20- to ~2.00-fold) ↑Serum leptin (~2.07-fold) ↓Serum FT4 PND 15 (~69% of control) and PND180 (~95% of control) ↑Serum TSH PND15 (~1.29-fold) ↓Serum TSH PND21 (67% of control), PND90 (90% of control), and PND180 (69% of control) ↓Liver D1 activity PND15 (71% of control) and PND180 (50% of control) <b>No effect findings:</b> Maternal body weight and food consumption; F1 food consumption; F1 lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoproteins, and triglycerides) at 180 days of age

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**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Oliveira <i>et al.</i> 2010b)</b> Wistar rat dams received nicotine by subcutaneous osmotic mini pump infusion at 0 (saline) or 6 mg/kg bw/day from PND2 through PND15. Mean cotinine levels on PND15 were ~235 ng/mL in milk, ~245 ng/mL in maternal serum, and ~20 ng/mL in pup serum. Dams and pups were killed on PND15 and PND21.</p>	<p>10 dams/ 60 male pups per group</p>	<p>6, mini-pump</p>	<p><u>DAMS</u></p> <ul style="list-style-type: none"> <li>↓ Maternal food consumption during recovery: PND19 – PND21 (81% – 64% of control)</li> <li>↑ Maternal serum leptin (1.68-fold)</li> <li>↑ Milk leptin (4-fold)</li> <li>↑ Maternal serum PRL (1.60-fold)</li> <li>↑ Milk production (1.45-fold)</li> <li>↑ Milk energy (1.36-fold)</li> <li>↑ Lactose (1.29-fold)</li> <li>↑ Serum high-density lipoprotein cholesterol HDL-C at PND 15 (1.16-fold)</li> </ul> <p><u>PUPS</u></p> <ul style="list-style-type: none"> <li>↑ Serum leptin on PND 15 (1.36-fold)</li> <li>↑ Total fat mass on PND 15 (1.30-fold)</li> <li>Increased visceral fat mass (VFM) on PND15 (1.73-fold)</li> <li>↑ Total body protein on PND 21 (1.33-fold)</li> <li>↑ Serum corticosterone PND15 (~1.60-fold)</li> <li>↑ Adrenal catecholamine content (1.69-fold)</li> <li>↑ Adrenal gland mass (1.40-fold)</li> <li>↑ Adrenal TH protein expression (1.33-fold)</li> </ul> <p><b>No effect findings:</b> Maternal body weight, serum corticosterone, adrenal catecholamine, or adrenal mass; maternal total cholesterol, low-density lipoprotein cholesterol, very low-density lipoproteins, and triglycerides at PND 15 and lactating PND 21; pup body weight gain.</p>

**Appendix Tables**  
(version updated January 3, 2011)

**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Santos-Silva <i>et al.</i> 2010)</b> Wistar rat dams received nicotine by subcutaneous osmotic mini pump infusion at 0 (saline) or 6 mg/kg bw/day from PND2 through PND16. Offspring were collected on PND15 and 180 days of age.</p> <p>Mean cotinine levels in milk, 225.8 ng/mL and 20.4 ng/mL cotinine levels in pup serum.</p> <p><u>Also presented in study, but not summarized here:</u> Protein levels of leptin signaling pathway components in the hypothalamus and thyroid;</p>	<p>10 dams/ 60 male pups per group</p>	<p>6, sc mini-pump</p>	<p>PND 15</p> <p>↑Visceral fat mass (1.75 fold) ↓Serum T3 and T4 (66% and 72% of control) ↑TSH (1.39-fold) ↑leptin (1.44-fold) ↓pituitary JAK-2 protein in pituitary (48% of control) ↑ hypothalamic levels of OB-R (1.58-fold); ↑ pSTAT-3 (1.34-fold); and ↓ pSTAT-3/STAT-3 ratio ↓ thyroid content of OB-R (56% of control); ↓JAK-2 (50% of control); ↓STAT-3 (53% of control); ↑pSTAT-3 (1.80-fold); ↑pSTAT-3/STAT-3 ratio</p> <p>180 days of age</p> <p>↑Pup body weight (1.1-fold) ↑Visceral fat mass (1.27-fold) ↑leptin (2.03-fold) ↓TSH (60% of control) ↓Serum T3 and T4 (72% and 89% of control) ↓ hypothalamic levels of OB-R (39% of control); ↓JAK-2 (58% of control); ↓pSTAT-3 (44% of control); and ↓ pSTAT-3/STAT-3 ratio ↑ thyroid content of OB-R (1.54-fold); ↓ pSTAT-3 (66% of control)</p> <p><b>No effect findings:</b> food intake during development; protein expression of Ob-R, STAT-3 or pSTAT-3 in pituitary</p>

**Appendix Tables**  
(version updated January 3, 2011)

**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Somm <i>et al.</i> 2008)</b> Pregnant Sprague-Dawley OAF rats received nicotine hydrogen tartrate by subcutaneous osmotic mini pump infusion at 0 (saline) or 3 mg/kg bw/day from GD4 to GD17. Mean serum levels of cotinine and other nicotine metabolites were 281 ng/mL on GD5 and 252 mg/mL on GD19. F1 males: Pancreas and adipose tissue were collected for histopathological and immunohistochemical examination on PND7; epididymal white adipose tissue (WAT) was examined on PND21; pancreatic mRNA was quantified, pancreatic and blood insulin were determined; acute cold exposure test conducted at 15 weeks; calorimetry and physical activity were assessed at 18 weeks; IPGTT and ITT were conducted at 26 weeks.</p> <p><u>Also presented in study, but not summarized here:</u> Experiments with isolated Islets of Langerhans. Judy, go ahead and add Kris-What would like me to capture Figure 4 B mRNA? Not sure-</p>	<p>5 – 10 F1 males per group</p>	<p>3, sc mini-pump</p>	<p><u>PND7 Pancreas (histo- and immunohistochemistry)</u>            ↓Ratio endocrine to whole pancreas (69% of control)            ↓Islet size (81% of control)            ↓ Number of islets/ section (56% of control)            ↓Expression of transcription factors Pax-6 and Nkx6.1            ↓Insulin and glucagon mRNA            ↓Expression Kir6.2, glut 2, and glucokinase mRNA            ↓Expression of survivin (confirmed by immunohistochemistry)</p> <p><u>At Weaning (adipose tissue analysis)</u>            ↑Pup body weight (~1.07)            ↑Relative weight, epididymal white adipose tissue (~1.52-fold)            ↓Relative weight, brown adipose tissue (~71% of control)            ↑Size of adipocytes (1.38-fold)            ↑Expression of C / EBP-α, PPAR-γ, SREBP-1C, aP2 and adipsin            ↑mRNA expression for leptin (9.1-fold), resistin (3.3-fold), apelin (4.2-fold), LPL (2.08-fold)</p> <p><u>At 18 – 20 weeks (calorimetry and physical activity)</u>            ↑Body weight – on normal diet (~1.17-fold) and also on high fat diet (~1.07-fold)            ↑Relative weight, epididymal white adipose tissue - on normal diet (1.31-fold) and also on high fat diet (1.53-fold)            ↑Relative weight, retroperitoneal white adipose tissue – on high fat diet (1.50-fold)            ↑Food efficiency – these rats needed less food (90% of controls) to gain one gram of body weight            ↑Short-term cold sensitivity (2.03 – 2.52-fold)            ↓Spontaneous ambulatory activity (76% of control during dark cycle, 79% of control during light cycle)</p> <p><u>At 26 Weeks (Glucose and Insulin Tolerance)</u>            ↑Glucose AUC (1.26-fold)            ↑Insulin AUC (1.11-fold)            ↑Expression of adiponectin (2.23-fold) and resistin (2.00-fold) in epididymal white adipose tissue</p> <p><b>No effect findings:</b> Maternal body weight gain, feed and water consumption; pup body weight at birth; food consumption and energy intake, basal energy expenditure at 18-20 weeks; basal glucose and insulin at 26 weeks.</p>

**Appendix Tables**  
(version updated January 3, 2011)

**Appendix Table B. Summary of Animal Studies of Developmental Exposure to Nicotine**

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Effects
<p><b>(Williams and Kanagasabai 1984)</b> Pregnant Sprague-Dawley rats received nicotine tartrate in their drinking water at 0 (water) or 0.120mg/mL (~2.46 mg/kg-d) from GD0 – GD20. Average daily dose was estimated to be ~2.46 mg/kg/day. Dams were killed on GD20, fetuses and placentas were weighed, body composition was analyzed for the fetuses.</p>	<p>12 pregnant females/group</p>	<p>~2.46, drinking water</p>	<p>↓ Maternal body weight gain during week 1 (43% of control), week 2 (74% of control), and entire pregnancy (77% of control)            ↑ Maternal basal lipolysis (1.70-fold)            ↑ Maternal stimulated lipolysis (1.32-fold)            ↑ Fetal body fat (mg/fetus) (1.51-fold)</p> <p><b>No effect findings:</b> Maternal feed consumption, litter size, lipogenesis; fetal weight, fetal DNA, fetal protein, placental weight, % body water</p>

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