Elsevier Editorial System(tm) for Paediatric Respiratory Reviews

Manuscript Draft

Manuscript Number: YPRRV-D-16-00045

Title: Diabetes in pregnancy and lung health in offspring: developmental origins of respiratory disease

Article Type: Commissioned Review

Keywords: maternal diabetes, gestational diabetes, pregnancy, developmental origins of health and disease, lung development, asthma, chronic lung disease, respiratory distress syndrome

Corresponding Author: Dr Meghan Azad, PhD

Corresponding Author's Institution:

First Author: Meghan Azad, PhD

Order of Authors: Meghan Azad, PhD

Diabetes in pregnancy and lung health in offspring: developmental origins of respiratory disease

Azad MB[1,2*], Moyce BL[1,3], Guillemette L[1,4], Pascoe CD[1,5], Wicklow B[1,2], McGavock JM[1,2], Halayko AJ[1,5], Dolinsky VW[1,3].

1. Manitoba Developmental Origins of Chronic Diseases in Children Network (DEVOTION); Children's Hospital Research Institute of Manitoba, Winnipeg, Canada
2. Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada
3. Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada
4. Applied Health Sciences, University of Manitoba, Winnipeg, Canada
5. Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, Canada

*Corresponding author: Meghan Azad, PhD
Research Scientist, Children's Hospital Research Institute of Manitoba
Assistant Professor, Pediatrics & Child Health, University of Manitoba
501G - 715 McDermot Ave.
Winnipeg, MB Canada R3E 3P4
(204) 975-7754
meghan.azad@umanitoba.ca

Key words: maternal diabetes, gestational diabetes, pregnancy, developmental origins of health and disease, lung development, asthma, chronic lung disease, respiratory distress syndrome

Word count: 3429

Summary

Diabetes is an increasingly common complication of pregnancy. In parallel with this trend, a rise in chronic lung disease in children has been observed in recent decades. While several adverse health outcomes associated with exposure to diabetes *in utero* have been documented in epidemiological and experimental studies, few have examined the impact of diabetes in pregnancy on offspring lung health and respiratory disease. We provide a comprehensive overview of current literature on this topic, finding suggestive evidence that exposure to diabetes *in utero* may have adverse effects on lung development. Delayed lung maturation and increased risk of respiratory distress syndrome (RDS) have been consistently observed among infants born to mothers with diabetes and these findings are also observed in some rodent models of diabetes in pregnancy. Further research is needed to confirm and characterize epidemiologic observations that diabetes in pregnancy may predispose offspring to childhood wheezing illness and asthma. Parallel translational studies in human pregnancy cohorts and experimental models are needed to explore the role of fetal programming and other potential biological mechanisms in this context.

Educational aims:

1. Review existing evidence linking diabetes in pregnancy with lung development and respiratory health in offspring
2. Describe potential mechanisms for the association between diabetes in pregnancy and respiratory health in offspring
3. Acknowledge the strengths and limitations of epidemiologic studies and rodent models addressing the association between diabetes in pregnancy and lung health in offspring

Directions for Future Research

1. Long-term follow up studies monitoring respiratory health of children exposed to diabetes *in utero* are needed, since no studies to date have evaluated this association beyond early adolescence.
2. Epidemiologic associations between diabetes in pregnancy and lung health in offspring should be replicated and studied in rodent models, where molecular mechanisms can be investigated in detail in order to inform new treatments and prevention strategies.
3. All studies should clearly distinguish between pre-gestational (type 1 or type 2) and gestational diabetes arising in pregnancy since these exposures are not equivalent.

1 Introduction

Respiratory health is influenced by intrinsic and extrinsic environmental stressors during fetal and postnatal development, with important implications for chronic lung disease later in life (1). Diabetes in pregnancy has long been associated with adverse maternal and neonatal outcomes and has recently emerged as an important trigger for the fetal programming of lifelong metabolic and cardiovascular health outcomes in offspring (2). These clinical observations have been confirmed in rodent models, which have further identified specific alterations in gene expression among offspring exposed to diabetes *in utero* (reviewed in (2)). While the majority of this research has focused on short-term maternal and neonatal health outcomes, or long-term metabolic and cardiovascular consequences in offspring, there is a growing body of evidence suggesting an impact of diabetes in pregnancy on offspring lung development and respiratory health. We provide an overview of this literature and identify key knowledge gaps requiring additional research.

1.1 Maternal diabetes in pregnancy

Diabetes affects a rising proportion of pregnancies worldwide, including up to 10% in the United States (3). Dysregulation of glycemic control during pregnancy is associated with numerous adverse maternal and neonatal outcomes, including preeclampsia, preterm birth, macrosomia and stillbirth (4). Since the risk of harm for offspring increases with the duration and extent of hyperglycemia exposure, it is important to distinguish pre-gestational diabetes (type 1 diabetes (T1D) or type 2 diabetes (T2D) diagnosed prior to pregnancy) from gestational diabetes mellitus (GDM), defined as fasting or post-prandial hyperglycemia first detected during pregnancy (2). T1D is characterized by hyperglycemia due to an absolute deficiency of insulin production, whereas hyperglycemia in T2D and GDM is associated with both insulin resistance (in hepatic and/or peripheral tissues) and insufficient insulin secretion to maintain euglycemia (3). There is convincing evidence that exposure to diabetes earlier in pregnancy

(i.e. exposure to pre-gestational diabetes) carries the most severe health consequences for the offspring (4), but the distinction between pre-gestational diabetes and true GDM is rarely made in respiratory health studies. Currently the standard screening protocol to detect diabetes in pregnancy is an oral glucose challenge test between 24 and 28 weeks of gestation, which would not distinguish between GDM or undiagnosed pre-pregnancy T2D (5). Moreover, most health registries used in longitudinal research do not reliably distinguish between types of diabetes.

1.2 Fetal lung development

Fetal lung development occurs in five stages (**Figure 1**), beginning with tracheal separation from the esophagus in the embryonic stage at 3 weeks of gestation and ending with the development of mature alveoli in the alveolarization stage, which occurs following birth and extends into early childhood (6). The intermediate stages (pseudoglandular, canalicular, and saccular) encompass the development of the branched airway structure, epithelial lined sacs that become alveoli, and various layers of the airway and pulmonary vasculature walls (7). In addition, surfactant production begins at 24 weeks of gestation and continues until birth. Surfactant is a complex mixture of phospholipids and proteins that act to reduce surface tension in the alveoli and prevent alveolar collapse during expiration (8). Environmental exposures throughout gestation and postnatally can therefore have significant and distinct impacts on lung development and future health. Extensive research has been undertaken to establish how maternal nutrition (9), smoking (10), and exposure to air pollution (11) influence lung development and respiratory health; however, much less is known about how diabetes in pregnancy affects fetal lung development and subsequent respiratory health in the offspring.

2 Diabetes in pregnancy and neonatal respiratory outcomes

2.1 Respiratory Distress Syndrome

Respiratory distress syndrome (RDS) is an important cause of neonatal morbidity, affecting 40 000 infants each year in the US (12). RDS is characterized by a lack of functional surfactant in the neonatal lung, resulting in collapse of the terminal air spaces. Treatment involves ventilation and oxygen therapy which can damage the epithelium, resulting in plasma leakage into the collapsed airspaces and formation of a fibrin-rich hyaline membrane that further damages the tissue (13). Infants who are treated for RDS, and especially those who suffer damage from the treatment, are more likely to develop asthma (14), putting them at increased risk for lifelong chronic lung disease.

Preterm birth is the strongest risk factor for RDS (15), and women with pre-gestational diabetes or GDM are more likely to deliver preterm (16, 17). Additionally, diabetes in pregnancy may be an independent risk factor for RDS (**Table 1**). A 1976 cohort study by Robert et al. including over 10 000 US children reported a 23.4% incidence of RDS among infants born to women with diabetes in pregnancy, compared to 1.3% among their counterparts without diabetes (18). This association persisted after controlling for gestational age at birth and additional confounders (relative risk (RR) 5.6, $p<0.0001$), although no distinction was made between pre-gestational diabetes and GDM. More recent studies reveal that RDS risk is particularly elevated following exposure to pre-gestational (19) or insulin-treated (20) diabetes in pregnancy. However, this is not a universal finding as two studies in very low birthweight infants found no association between diabetes in pregnancy and RDS (21, 22). These conflicting results may be due to differences in gestational age, mode of delivery, maternal blood glucose control, prenatal steroid use, or RDS definitions between studies.

The mechanism by which diabetes in pregnancy could increase the risk for RDS is related to the composition and integrity of pulmonary surfactant in the developing fetus. Specifically, expression of

Comment [HR1]: RDS is not an easily identified risk factor for asthma. As recently reviewed (Kouzouna A, Gilchrist FJ, Ball V, Kyriacou T, Henderson J, Pandyan AD, Lenney W. A systematic review of early life factors which adversely affect subsequent lung function. Paediatr Respir Rev. 2016 Mar 14. pii: S1526-0542(16)00030-0. doi: 10.1016/j.prrv.2016.03.003.), BPD and mechanical ventilation have the stronger association with asthma. It might be better to say – infants who are treated for RDS, and especially those who suffer damage from the treatment are more likely … and then use the reference above instead of the one from 1996

Comment [MA2]: Changed as suggested. Thanks!

surfactant proteins B and C in epithelial cell culture is inhibited by insulin (23, 24), which is commonly elevated among neonates exposed to hyperglycemia during pregnancy (25)**.** In addition, pregnancy complicated by diabetes is associated with delayed appearance of phosphatidylglycerol, a major lipid component of surfactant and an important marker of fetal lung maturity (26). A study evaluating glycemic control in 621 mothers with diabetes found that smaller gestational age at birth and poor maternal glycemic control independently predicted the delayed appearance of phosphatidylglycerol within neonatal surfactant (27). Another study found that well-controlled diabetes in pregnancy (T1D or GDM) does not delay fetal lung maturity (28), emphasizing the importance of maternal glycemic control for maintaining normal fetal lung development.

2.2 Bronchopulmonary Dysplasia

Bronchopulmonary Dysplasia (BPD) is a chronic lung condition characterized by thin alveoli septa and interstitial thickening that can result from extended ventilator use in preterm infants, often for the treatment of RDS. Despite the association of diabetes in pregnancy with preterm birth and RDS (described above), a Swedish study of over 100 000 preterm infants by Eriksson et al. found that both pre-gestational diabetes and GDM were associated with *reduced* risks of BPD (odds ratio (OR) for pre-gestational diabetes: 0.64; 95% confidence interval (95%CI) 0.42-0.97; OR for GDM 0.36; 95%CI 0.20-0.65) (29). This counterintuitive protective association was not explained by insulin use among mothers with diabetes, or higher birthweight for gestational age among their infants. Prenatal steroid use was not considered. The authors speculated that diabetes in pregnancy might initiate fetal stress reactions and increase endogenous corticosteroid levels, thus promoting lung maturation in the newborn infant. Bental and colleagues' study of very low birthweight infants in Israel also found a small reduction in BPD risk among infants born to mothers with GDM or pre-gestational diabetes (19.9% vs. 24.5%, p=0.002); however, this association was not significant after adjusting for confounders (OR 1.00; 95%CI 0.81-1.25).

They concluded that the apparent protective association was due to increased use of prenatal steroids among mothers with diabetes, and increased birthweight among their infants (30). Similarly, a matched double-cohort study in very low birthweight Canadian infants by Rehan et al. found no association between diabetes in pregnancy and BPD (22), and a study of nearly 12 000 very low birthweight infants across six South American countries found no significant association after adjusting for birthweight, prenatal steroids, and other confounders (OR 1.20; 95%CI 0.91-1.58) (21). Therefore, current evidence for an independent association between diabetes in pregnancy and BPD in offspring is weak.

2.3 Congenital Diaphragmatic Hernia

Congenital Diaphragmatic Hernia (CDH) is a rare birth defect in which the diaphragm fails to fully develop, allowing the contents of the abdomen to enter the chest cavity. This disease is associated with severely underdeveloped (hypoplastic) lungs, the latter effect occurring independently of diaphragm abnormalities (31). Children born with CDH surviving surgical repair carry chronic lung defects including increased parenchymal elastance and reduced diffusing capacity, which severely compromises their respiratory health and quality of life. In a cohort study of 492 infants with CDH and 4900 controls, maternal pre-gestational diabetes was strongly associated with CDH (OR 12.53; 95%CI: 2.40-65.43) (32). A similar association was found by Correa et al. in a larger study of 17 000 infants (OR 4.70, 95%CI: 1.02-21.60) (33). These studies did not distinguish between T1D and T2D or evaluate the possible impact of GDM. The underlying mechanism for this association has not been studied directly, but the authors hypothesized that it may involve dysregulation of genes responsible for apoptosis and organogenesis during fetal development, suggesting an association between maternal metabolic health and development of lung-associated abnormalities in CDH (32).

3 Diabetes in pregnancy and respiratory outcomes in infancy and childhood

3.1 Wheezing

Wheezing is common during infancy and early childhood, with 20% to 50% of infants experiencing at least one episode in the first year of life across different settings (34, 35). A recent pooled analysis of individual participant data from 14 European birth cohorts (n=85 509) found that diabetes in pregnancy (pre-gestational diabetes, GDM, and glucose intolerance in pregnancy) was not associated with parent-reported wheezing in offspring from birth to 24 months of age (36) (**Table 2**). Specifically, after adjusting for confounders (e.g. maternal smoking, education, and asthma) and other pregnancy complications (overweight/obesity and hypertensive disorders), the authors found no consistent association between diabetes in pregnancy and "ever wheezing" (Relative Risk (RR) 1.04; 95% CI 0.97–1.13) or "recurrent wheezing" (RR 1.24; 95% CI 0.86–1.79). However, there was evidence of heterogeneity across cohorts (p=0.03), which the authors attributed to variations in diagnostic criteria, screening policies and actual prevalence of diabetes in pregnancy between countries.

In a cross-sectional study of over 15 000 Italian children, Rusconi et al. evaluated persistent wheezing later in childhood (age 6-7 years) and found a significant association with diabetes in pregnancy (37). Children born to a mother with GDM or pre-gestational diabetes were significantly more likely to have persistent wheezing by school age (OR 1.84; 95% CI: 1.06-3.20), after adjustment for low birthweight, socioeconomic status, maternal age and smoking, and other confounders. These findings could signal long-term respiratory consequences of exposure to diabetes *in utero*, since we and others have found that wheezing in early childhood predicts significantly reduced lung function and increased asthma risk in adolescence (38, 39).

3.2 Asthma

Asthma is the most common chronic disease of childhood, affecting 9.3% of American children (40) and costing over $56 billion annually in the United States alone (41). International reports have estimated asthma prevalence at 14% among 6-14 year old children worldwide (42). The developmental origins of asthma have been intensely studied since prevention is regarded as the best approach to managing the health and economic burden associated with this disease. However, only four studies have specifically investigated the association between diabetes in pregnancy and childhood asthma (43-46) (**Table 2**). All found a positive association, although none distinguished between GDM and pre-gestational diabetes. In the smallest cohort study by Risnes et al. (n=1401 US children) (45), diabetes in pregnancy was associated with a three-fold increased risk of physician-diagnosed asthma by 6 years of age (OR 3.63; 95% CI 1.46-9.04); however, this estimate was not adjusted for any confounders since diabetes in pregnancy was not the primary exposure of interest in this study. A significant but more modest association was reported by Aspberg et al. in a large population-based registry study of over 1 million Swedish children (44), in which the odds of hospitalization for asthma was 19% higher among children exposed to diabetes in pregnancy (OR 1.19; 95% CI: 1.12-1.28) after adjustment for maternal age, parity, smoking, and other confounders. Similarly, in a national Finnish database study of 1.2 million children (46), Haataja et al. found that moderately preterm infants (32 -34 weeks gestational age at birth) exposed to diabetes in pregnancy were at increased risk of physician-diagnosed asthma by 7 years old (hazard ratio (HR) 1.62, 95% CI: 1.02-2.58), independent of many confounders including delivery mode, maternal age, antenatal steroids, and maternal smoking. This relationship was not found for other preterm infants, and although it was nearly significant in term infants, the effect was small (HR 1.09; 95%CI 0.99-1.21).

We performed a cross-sectional study in 3574 Canadian school-aged children (43), finding that those with parent-reported asthma were more likely to have mothers (2.9 vs 1.2%, p=0.003) but not fathers (1.4 vs 1.3%, p=0.89) with diabetes. This finding is consistent with the fetal programming hypothesis (47), suggesting that a hyperglycemic intrauterine environment increases the risk for asthma in childhood. Interestingly, our study further showed that diabetes in pregnancy did not confer a strong independent risk for asthma; rather, this exposure amplified the effects of maternal asthma and environmental tobacco smoke (ETS). For example, diabetes in pregnancy increased the ETS-associated risk for asthma from 1.4-fold (OR 1.40; 95%CI 1.13–1.73) to 5.7-fold (OR 5.68; 95%CI 1.18–27.36; P for interaction= 0.08). Based on these findings, we proposed that fetal hypoxia and immune dysregulation may be synergistically amplified in pregnancies complicated by diabetes in combination with maternal asthma or ETS exposure, leading to a markedly elevated risk of asthma in offspring (43). Both fetal hypoxia and immune dysregulation are established mechanisms for asthma development (48, 49) and they have been independently linked to diabetes in pregnancy (50, 51). Others have framed asthma as an autoimmune disease arising from immunological disturbances that are imprinted during fetal life (44), perhaps involving factors common to T1D, which is also an autoimmune disorder. However, these postulated mechanisms and interactions remain to be replicated in other cohorts and proven in experimental models.

3.3 Other respiratory issues

Kumar et al. found that GDM increased the risk of allergic sensitization by 3 years of age in full term (OR 6.05; 95%CI 1.17-31.18), but not preterm (OR 0.33; 95%CI 0.07-1.60) offspring (52). These effects were independent of family history, infant sex, maternal BMI, race and education, breastfeeding, peripartum antibiotic use, and c-section delivery. These findings may be relevant to respiratory health since allergic sensitization by 2 years of age is a strong risk factor for wheezing throughout childhood (38, 53). The

authors speculated that the lack of association in preterm infants might be due to the shorter exposure to hyperglycemia in GDM.

4 Evidence from animal models

The epidemiologic studies described above provide intriguing observational evidence that exposure to diabetes *in utero* may be associated with impaired lung development and poor respiratory health. However, human studies are limited in their ability to characterize gestational exposures, control for confounding factors, perform lifelong follow up, and study biological mechanisms. These limitations can be addressed in animal models, which allow precise control of environmental factors and detailed mechanistic studies to provide insight into the human condition. Similar to humans, rodent lung development begins with the origination of lung buds, lobular division, airway branching and bronchiolar development during gestation, and continues postnatally with secondary septation and expansion of the number and size of capillaries and alveoli (54) (**Figure 1**). In contrast to humans where alveolar duct and air sac development occurs entirely *in utero*, this process continues for several days after birth in mice. Keeping this difference in mind, rodents provide a useful and appropriate model for human lung development.

4.1 Rodent models of pre-gestational diabetes and GDM

Several rodent systems have been developed to model T1D, T2D and GDM using drug administration, genetic modification, and dietary interventions. Notably, these methods and the resulting hyperglycemia can impact fertility, presenting specific challenges for studies of pregnancy.

The most commonly used rodent model of diabetes in pregnancy is streptozotocin or alloxan administration prior to pregnancy to extinguish the insulin secretion capacity of pancreatic beta cells in order to induce T1D (55). Genetic models include the non-obese diabetic (NOD) mouse and the bio-

breeding (BB) rat that spontaneously develop T1D via autoimmune attack, much like human T1D (56). Established genetic models of T2D include the leptin-deficient ob/ob mouse and the leptin-resistant db/db mouse; however, these are not useful models of T2D in pregnancy since these homozygous mice are infertile (55). Several approaches have been used to model GDM. In db/+ or ob/+ heterozygotes, GDM occurs spontaneously when partial leptin deficiency is combined with the added metabolic stress of pregnancy (57). Alternatively, high fat or high carbohydrate diet-induced models can provide the necessary "pre-diabetic" conditions that will result in spontaneous GDM (2, 55). Diet-induced models have the benefits of avoiding genetic manipulations or drug-induced teratogenic effects, and of providing an ideal model to evaluate dietary interventions for GDM prevention and treatment.

4.2 Lung development in rodent models of diabetes in pregnancy

The above models of diabetes in pregnancy have been utilized extensively to study the programming of metabolic and cardiovascular health in the offspring (reviewed in (2)); however, relatively few studies have been performed to assess lung development in these model systems (**Table 3**).

Consistent with human studies (Section 2.1), delayed pneumocyte differentiation (58) and lung maturation (59-61) have been observed in rodent models of diabetes during pregnancy, including streptozotocin-induced T1D (60), genetic T1D in BB rats (61), and obesity-associated GDM in db/+ mice (59). Reduced content and synthesis of surfactant phospholipids (62-64) and surfactant proteins (65) have also been observed in neonatal rats born to dams with streptozotocin-induced T1D. Evidence from the db/+ model of GDM suggests that these surfactant defects occur as a consequence of dysregulated phospholipid synthesis or metabolism in the developing fetus (59). Consistent with clinical evidence (4), more severe abnormalities are observed in rodent offspring when exposure to diabetes is experienced early during embryonic organogenesis versus later in fetal development (55). Further studies using

rodent models are required to fully characterize the molecular mechanisms linking diabetes in pregnancy to impaired surfactant levels and lung development in the offspring.

4.3 Chronic respiratory disease in rodent offspring exposed to diabetes in pregnancy

There is a paucity of studies using animal models to directly investigate chronic respiratory disease following exposure to diabetes during fetal development. Since an association is apparent in epidemiologic studies (Section 3), there is a need to explore the underlying mechanisms that may link diabetes in pregnancy with chronic lung disease in experimental models. There is an established link between maternal hyperglycemia and placental inflammation, which could lead to impaired fetal lung development and higher risk for inflammatory disorders later in life (66). Indeed, dysregulated prostaglandin signalling has been linked to delayed fetal lung maturation in streptozotocin-treated mice (67) and alloxan-treated rabbits (68). While these models of T1D provide a good starting point for analyzing the impact of diabetes in pregnancy on lung health in offspring, GDM and pre-gestational T2D are more prevalent than T1D in clinical settings. Diet-induced models of T2D and GDM (2) will therefore be particularly helpful in determining the mechanisms by which exposure to diabetes *in utero* influences the development of chronic lung disease.

5 Conclusions and Future Research Directions

Diabetes is an increasingly common complication of pregnancy (3) and a parallel rise in chronic lung disease has been observed in recent decades (69). While the adverse metabolic consequences of exposure to diabetes *in utero* have been repeatedly documented in clinical and experimental studies (2), few have examined the potential impact of diabetes in pregnancy on lung health and respiratory disease in the offspring. From the limited literature on this topic, there is suggestive evidence of a clinically important association. Delayed lung maturation and increased risk of RDS have been consistently observed among infants born to mothers with diabetes and these findings are recapitulated in rodent

models of diabetes in pregnancy. Further research is needed to confirm and characterize observations that diabetes in pregnancy may predispose offspring to wheezing illness and childhood asthma, as the vast majority of these studies have been retrospective and none have distinguished between pre-gestational diabetes and GDM.

Long-term epidemiologic studies will be particularly valuable for establishing lifecourse implications of diabetes in pregnancy on lung health, as no study to date has investigated these associations beyond early adolescence. All future studies should clearly distinguish between GDM and pre-gestational T1D or T2D, since research to date clearly demonstrates that "diabetes in pregnancy" is not a homogeneous exposure. Rodent studies in appropriate models of diabetes in pregnancy will be essential for identifying biological mechanisms. In particular, parallel studies in human pregnancy cohorts and experimental models are needed to explore the role of fetal programming in this context.

6 Acknowledgements

The Developmental Origins of Chronic Diseases in Children Network (DEVOTION) is supported by Research Manitoba and the Lawson Foundation. MBA and AJH are members of the Canadian Respiratory Research Network and the AllerGen Network of Centres of Excellence. BLM is the recipient of a Research Manitoba studentship. LG is supported by a Fonds de Recherche en Santé – Québec PhD training award and the University of Manitoba Graduate Enhancement of Tri-Council Stipends program. CDP is the recipient of an Integrated and Mentored Pulmonary and Cardiovascular Training (IMPACT) post-doctoral fellowship. JMM holds a CIHR Applied Health Chair in Resilience and Obesity in Youth. AJH is supported by the Canada Research Chairs program. VWD is the Amgen-Stewart Whitman Young Investigator and the Dr. J.A. Moorhouse Fellow of the Diabetes Foundation of Manitoba.

7 Tables & Figure

Table 1. Summary of human studies reporting associations of diabetes in pregnancy respiratory outcomes in neonates.

Study	Population and Setting	Type of Diabetes in Pregnancy	Respiratory Outcome	Association
Robert et al. 1975 (18)	Retrospective cohort 10 957 newborns USA	Unspecified	Respiratory Distress Syndrome	Increased risk (RR 5.6, p<0.0001)
Abu-Heija et al. 2015 (19)	Retrospective chart review 5811 newborns Oman	Pre-gestational or GDM (separately)	Respiratory Distress Syndrome	Increased risk with pre-gestational vs. GDM (8.5% vs. 2.6%; p=0.03)
Becquet et al. 2015 (20)	Retrospective cohort 18 095 newborns France	Pre-gestational and GDM (combined)	Respiratory Distress Syndrome	Increased risk with insulin-treated diabetes (IRR 1.44; 95%CI 1.00 – 2.08) No association with untreated diabetes (IRR 0.95; 95%CI 0.68-1.32)
Rehan et al. 2002 (22)	Retrospective matched double-cohort 582 VLBW newborns Canada	Pre-gestational and GDM (combined)	Respiratory Distress Syndrome	No association (RR 0.70; 95%CI 0.35-1.42)
Grandi et al. 2015 (21)	Retrospective cohort 11 991 VLBW newborns South America	Pre-gestational and GDM (combined)	Respiratory Distress Syndrome	No association (OR 1.18; 95%CI 0.90-1.56)
Eriksson et al. 2014 (29)	Registry-based cohort 106 339 preterm infants Sweden	Pre-gestational or GDM (separately)	Bronchopulmonary Dysplasia	Reduced risk (pre-gestational: OR 0.64; 95%CI 0.42-0.97; GDM OR: 0.36; 95%CI 0.20-0.65)
Bental et al. 2011 (30)	Registry-based cohort 15 784 VLBW newborns Israel	Pre-gestational and GDM (combined)	Bronchopulmonary Dysplasia	No association (OR 1.00; 95%CI 0.81-1.25)
Rehan et al. 2002 (22)	Retrospective matched double-cohort 582 VLBW newborns Canada	Pre-gestational and GDM (combined)	Bronchopulmonary Dysplasia	No association (RR 1.24; 95%CI 0.59-2.61)
Grandi et al. 2015 (21)	Retrospective cohort 11 991 VLBW newborns South America	Pre-gestational and GDM (combined)	Bronchopulmonary Dysplasia	No association (OR 1.20; 95%CI 0.91-1.58)
McAteer et al. 2014 (32)	Registry-based case-control 492 newborns with CDH + 4920 controls USA	Pre-gestational (Type 1 or 2)	Congenital Diaphragmatic Hernia	Increased risk (OR 12.53; 95%CI 2.40-65.43)
Correa et al. 2008 (33)	Registry-based case-control 13 030 newborns with birth defects + 4895 controls USA	Pre-gestational (Type 1 or 2)	Congenital Diaphragmatic Hernia	Increased risk (OR 4.70; 95%CI 1.02-21.60)

CI, confidence interval; GDM, gestational diabetes mellitus; HR, hazard ratio; IRR, incidence rate ratio; OR, odds ratio; RR, relative risk; VLBW, very low birthweight.

Table 2. Summary of human studies reporting associations of diabetes in pregnancy and respiratory outcomes in infants and children.

Study	Population and Setting	Type of Diabetes in Pregnancy	Respiratory Outcome (age)	Association
Zugna et al. 2015 (36)	Meta-analysis 85 509 infants 14 European cohorts	Pre-gestational and GDM (combined)	Wheezing (age 2 years)	No association (ever wheezing: RR 1.04; 95% CI 0.97–1.13; recurrent wheezing: RR 1.24, 95% CI; 0.86–1.79)
Rusconi et al. 2007 (37)	Retrospective cohort 15 609 children Italy	Pre-gestational and GDM (combined)	Wheezing (age 6 years)	Increased risk (OR 1.84; 95% CI 1.06-3.20)
Kumar et al. 2009 (52)	Retrospective cohort 680 children USA	GDM	Allergic sensitization (age 3 years)	Increased risk in term infants only (OR 6.05; 95%CI 1.17-31.18)
Risnes et al, 2011 (45)	Prospective cohort 1401 children USA	Unspecified	Asthma (age 6 years)	Increased risk (OR 3.63; 95% CI 1.46-9.04)
Haataja et al. 2016 (46)	Registry-based cohort 1.1 million children Finland	Pre-gestational and GDM (combined)	Asthma (age 7 years)	Increased risk (mild preterm: HR 1.62; 95% CI 1.02-2.58; full term: HR 1.09; 95% CI 0.99-1.21)
Azad et al. 2013 (43)	Retrospective cohort 3574 children Canada	Unspecified	Asthma (age 7 years)	Increased risk and effect modification: synergistic effects with maternal asthma and environmental tobacco smoke
Aspberg et al. 2010 (44)	Registry-based cohort 1.3 million children Sweden	Pre-gestational and GDM (combined)	Asthma (age 12 years)	Increased risk (OR 1.19; 95% CI 1.12-1.28)

CI, confidence interval; GDM, gestational diabetes mellitus; HR, hazard ratio; IRR, incidence rate ratio; OR, odds ratio; RR, relative risk.

Table 3. Summary of evidence from animal models linking maternal diabetes in pregnancy with fetal lung development and lung health in offspring.

Type of Diabetes	Animal Model	Impact on respiratory health of offspring	Studies
Type 1 Diabetes	Streptozotocin-treated rats	Delayed differentiation of type II pneumocytes cells, decreased pneumocytes, reduced surfactant proteins, altered surfactant phospholipids	Gewolb et al. 1985 (60), Rieutort et al. 1986 (62), Singh et al. 1983 (63), Niijar et al. 1984 (64), Moglia et al. 1996 (65) Treviño-Alanís et al. 2009 (58)
	Alloxan-treated rabbits	Reduced prostaglandin E2 affecting organogenesis	Tsai et al. 1982 (68)
	Bio-Breeding (BB) rats	Delayed lung maturation, reduced insulin receptor expression on lung tissue	Mulay et al. 1983 (61)
	Non-obese diabetic (NOD) mice	Unknown	N/A
Type 2 Diabetes	Leptin-deficient ob/ob mice; Leptin-resistant db/db mice	Unknown (infertile)	N/A
Gestational Diabetes	Leptin-deficient db/+ mice	Delayed lung maturation, altered pulmonary phospholipid expression	Lawrence et al. 1989 (59)
	Diet-induced GDM	Unknown	N/A

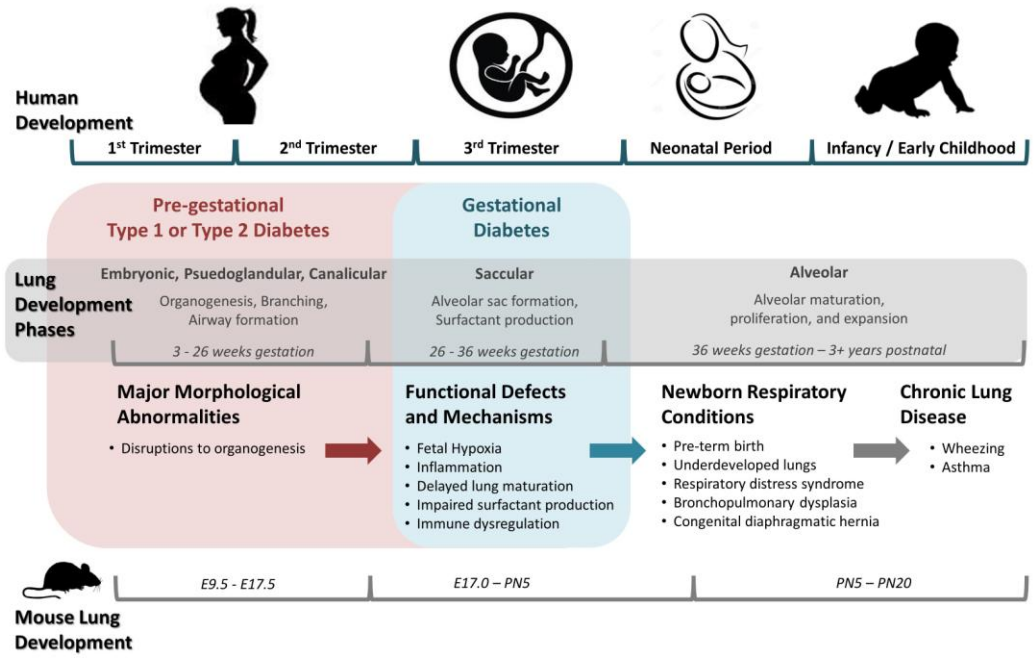

Figure 1. Diabetes in pregnancy and lung health in offspring. Timeline of human and mouse lung development overlapping with *in utero* exposure to maternal pre-gestational or gestational diabetes, and potential respiratory outcomes at birth and during early childhood. E=embryonic; PN=postnatal.

8 References

1. Manuck TA, Levy PT, Gyamfi-Bannerman C, Jobe AH, Blaisdell CJ. Prenatal and Perinatal Determinants of Lung Health and Disease in Early Life: A National Heart, Lung, and Blood Institute Workshop Report. *JAMA Pediatr*. 2016.
2. Pereira TJ, Moyce BL, Kereliuk SM, Dolinsky VW. Influence of maternal overnutrition and gestational diabetes on the programming of metabolic health outcomes in the offspring: experimental evidence. *Biochem Cell Biol*. 2014:1-14.
3. Reece EA, Leguizamon G, Wiznitzer A. Gestational diabetes: the need for a common ground. *Lancet*. 2009;373(9677):1789-1797.
4. Sweeting AN, Ross GP, Hyett J, Molyneaux L, Constantino M, Harding AJ, et al. Gestational Diabetes Mellitus in Early Pregnancy: Evidence for Poor Pregnancy Outcomes Despite Treatment. *Diabetes Care*. 2016;39(1):75-81.
5. Donovan L, Hartling L, Muise M, Guthrie A, Vandermeer B, Dryden DM. Screening Tests for Gestational Diabetes: A Systematic Review for the U.S. Preventive Services Task Force. *Annals of Internal Medicine*. 2013;159(2):115-122.
6. Zeltner TB, Caduff JH, Gehr P, Pfenninger J, Burri PH. The postnatal development and growth of the human lung. I. Morphometry. *Respir Physiol*. 1987;67(3):247-267.
7. Warburton D, El-Hashash A, Carraro G, Tiozzo C, Sala F, Rogers O, et al. Lung Organogenesis. *Current Topics in Developmental Biology*. 2010;90:73-158.
8. Nkadi PO, Merritt TA, Pillers DA. An overview of pulmonary surfactant in the neonate: genetics, metabolism, and the role of surfactant in health and disease. *Mol Genet Metab*. 2009;97(2):95-101.
9. Mayor RS, Finch KE, Zehr J, Morselli E, Neinast MD, Frank AP, et al. Maternal high-fat diet is associated with impaired fetal lung development. *American Journal of Physiology Lung Cellular and Molecular Physiology*. 2015;309:L360-368.
10. DiFranza JR, Aligne CA, Weitzman M. Prenatal and Postnatal Environmental Tobacco Smoke Exposure and Children's Health. *Pediatrics*. 2004;113:1007-1015.
11. Brauer M, Hoek G, Smit HA, Jongste JCd, Gerritsen J, Postma DS, et al. Air pollution and development of asthma, allergy and infections in a birth cohort. *European Respiratory Journal*. 2007;29:879-888.
12. Kamath BD, Macguire ER, McClure EM, Goldenberg RL, Jobe AH. Neonatal mortality from respiratory distress syndrome: lessons for low-resource countries. *Pediatrics*. 2011;127(6):1139-1146.
13. Ainsworth SB. Pathophysiology of neonatal respiratory distress syndrome: implications for early treatment strategies. *Treat Respir Med*. 2005;4(6):423-437.
14. Kouzouna A, Gilchrist FJ, Ball V, Kyriacou T, Henderson J, Pandyan AD, Lenney W. A systematic review of early life factors which adversely affect subsequent lung function. *Paediatr Respir Rev.* 2016. doi: 10.1016/j.prrv.2016.03.003. [Epub ahead of print]
15. Dani C, Reali Mf, Bertini G, Wiechmann L, Spagnolo A, Tangucci M, et al. Risk factors for the development of respiratory distress syndrome and transient tachypnoea in newborn infants. *European Respiratory Journal*. 1999;14:155-159.
16. Hedderson MM, Ferrara A, Sacks DA. Gestational diabetes mellitus and lesser degrees of pregnancy hyperglycemia: association with increased risk of spontaneous preterm birth. *Obstet Gynecol*. 2003;102(4):850-856.
17. Yang J, Cummings EA, O'Connell C, Jangaard K. Fetal and neonatal outcomes of diabetic pregnancies. *Obstet Gynecol*. 2006;108(3 Pt 1):644-650.

18. Robert MF, Neff RK, Hubbell JP, Taeusch HW, Avery ME. Association between maternal diabetes and the respiratory-distress syndrome in the newborn. *The New England Journal of Medicine*. 1976;294:357-360.
19. Abu-Heija AT, Al-Bash M, Mathew M. Gestational and Pregestational Diabetes Mellitus in Omani Women: Comparison of obstetric and perinatal outcomes. *Sultan Qaboos Univ Med J*. 2015;15(4):e496-500.
20. Becquet O, El Khabbaz F, Alberti C, Mohamed D, Blachier A, Biran V, et al. Insulin treatment of maternal diabetes mellitus and respiratory outcome in late-preterm and term singletons. *BMJ Open*. 2015;5(6):e008192.
21. Grandi C, Tapia JL, Cardoso VC. Impact of maternal diabetes mellitus on mortality and morbidity of very low birth weight infants: a multicenter Latin America study. *J Pediatr (Rio J)*. 2015;91(3):234-241.
22. Rehan VK, Moddemann D, Casiro OG. Outcome of very-low-birth-weight (< 1,500 grams) infants born to mothers with diabetes. *Clin Pediatr (Phila)*. 2002;41(7):481-491.
23. Miakotina OL, Dekowski SA, Snyder JM. Insulin inhibits surfactant protein A and B gene expression in the H441 cell line. *Biochimica Et Biophysica Acta*. 1998;1442:60-70.
24. Miakotina OL, Goss KL, Snyder JM. Insulin utilizes the PI 3-kinase pathway to inhibit SP-A gene expression in lung epithelial cells. *Respiratory Research*. 2002;3:27.
25. Westgate JA, Lindsay RS, Beattie J, Pattison NS, Gamble G, Mildenhall LF, et al. Hyperinsulinemia in cord blood in mothers with type 2 diabetes and gestational diabetes mellitus in New Zealand. *Diabetes Care*. 2006;29(6):1345-1350.
26. Leung-Pineda V, Gronowski AM. Biomarker tests for fetal lung maturity. *Biomarkers in Medicine*. 2010;4:849-857.
27. Piper JM, Xenakis EM, Langer O. Delayed appearance of pulmonary maturation markers is associated with poor glucose control in diabetic pregnancies. *The Journal of Maternal-Fetal Medicine*. 1998;7:148-153.
28. Piazze JJ, Anceschi MM, Maranghi L, Brancato V, Marchiani E, Cosmi EV. Fetal lung maturity in pregnancies complicated by insulin-dependent and gestational diabetes: a matched cohort study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*. 1999;83:145-150.
29. Eriksson L, Haglund B, Odlind V, Altman M, Kieler H. Prenatal inflammatory risk factors for development of bronchopulmonary dysplasia. *Pediatric Pulmonology*. 2014;49:665-672.
30. Bental Y, Reichman B, Shiff Y, Weisbrod M, Boyko V, Lerner-Geva L, et al. Impact of Maternal Diabetes Mellitus on Mortality and Morbidity of Preterm Infants (24–33 Weeks' Gestation). *Pediatrics*. 2011;128:e848-e855.
31. Arkovitz MS, Hyatt BA, Shannon JM. Lung development is not necessary for diaphragm development in mice. *Journal of Pediatric Surgery*. 2005;40:1390-1394.
32. McAteer JP, Hecht A, De Roos AJ, Goldin AB. Maternal medical and behavioral risk factors for congenital diaphragmatic hernia. *Journal of Pediatric Surgery*. 2014;49:34-38; discussion 38.
33. Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, et al. Diabetes mellitus and birth defects. *American Journal of Obstetrics and Gynecology*. 2008;199:237.e231-237.e239.
34. Garcia-Marcos L, Mallol J, Sole D, Brand PL. International study of wheezing in infants: risk factors in affluent and non-affluent countries during the first year of life. *Pediatr Allergy Immunol*. 2010;21(5):878-888.
35. Wood RA, Bloomberg GR, Kattan M, Conroy K, Sandel MT, Dresen A, et al. Relationships among environmental exposures, cord blood cytokine responses, allergy, and wheeze at 1 year of age in an inner-city birth cohort (Urban Environment and Childhood Asthma study). *J Allergy Clin Immunol*. 2011;127(4):913-919 e911-916.

36. Zugna D, Galassi C, Annesi-Maesano I, Baiz N, Barros H, Basterrechea M, et al. Maternal complications in pregnancy and wheezing in early childhood: a pooled analysis of 14 birth cohorts. *Int J Epidemiol*. 2015;44(1):199-208.
37. Rusconi F, Galassi C, Forastiere F, Bellasio M, De Sario M, Ciccone G, et al. Maternal Complications and Procedures in Pregnancy and at Birth and Wheezing Phenotypes in Children. *American Journal of Respiratory and Critical Care Medicine*. 2007;175:16-21.
38. Azad MB, Chan-Yeung M, Chan ES, Dytnerski AM, Kozyrskyj AL, Ramsey CD, et al. Wheezing patterns in early childhood and the risk of respiratory and allergic disease in adolescence. *JAMA Pediatr*. 2016;In Press.
39. Henderson J, Granell R, Heron J, Sherriff A, Simpson A, Woodcock A, et al. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. *Thorax*. 2008;63(11):974-980.
40. Moorman JE, Zahran H, Truman BI, Molla MT. Current asthma prevalence - United States, 2006-2008. *MMWR Suppl*. 2011;60(1):84-86.
41. Barnett SB, Nurmagambetov TA. Costs of asthma in the United States: 2002-2007. *J Allergy Clin Immunol*. 2011;127(1):145-152.
42. Global Asthma Network. The Global Asthma Report 2014. Aukland, New Zealand2014.
43. Azad MB, Becker AB, Kozyrskyj AL. Association of maternal diabetes and child asthma. *Pediatric Pulmonology*. 2013;48(6):545-552.
44. Aspberg S, Dahlquist G, Kahan T, Källén B. Confirmed association between neonatal phototherapy or neonatal icterus and risk of childhood asthma. *Pediatric Allergy and Immunology*. 2010;21:e733-e739.
45. Risnes KR, Belanger K, Murk W, Bracken MB. Antibiotic exposure by 6 months and asthma and allergy at 6 years: Findings in a cohort of 1,401 US children. *Am J Epidemiol*. 2011;173(3):310-318.
46. Haataja P, Korhonen P, Ojala R, Hirvonen M, Paassilta M, Gissler M, et al. Asthma and atopic dermatitis in children born moderately and late preterm. *Eur J Pediatr*. 2016.
47. Capra L, Tezza G, Mazzei F, Boner AL. The origins of health and disease: the influence of maternal diseases and lifestyle during gestation. *Ital J Pediatr*. 2013;39:7.
48. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat Immunol*. 2010;11(7):577-584.
49. Kindlund K, Thomsen SF, Stensballe LG, Skytthe A, Kyvik KO, Backer V, et al. Birth weight and risk of asthma in 3-9-year-old twins: exploring the fetal origins hypothesis. *Thorax*. 2010;65(2):146-149.
50. Nelson SM, Sattar N, Freeman DJ, Walker JD, Lindsay RS. Inflammation and endothelial activation is evident at birth in offspring of mothers with type 1 diabetes. *Diabetes*. 2007;56(11):2697-2704.
51. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*. 2015;36(7):709-715.
52. Kumar R, Ouyang F, Story RE, Pongracic JA, Hong X, Wang G, et al. Gestational diabetes, atopic dermatitis, and allergen sensitization in early childhood. *J Allergy Clin Immunol*. 2009;124(5):1031-1038 e1031-1034.
53. Lodge CJ, Zaloumis S, Lowe AJ, Gurrin LC, Matheson MC, Axelrad C, et al. Early-life risk factors for childhood wheeze phenotypes in a high-risk birth cohort. *J Pediatr*. 2014;164(2):289-294 e281-282.
54. Maeda Y, Dave V, Whitsett JA. Transcriptional control of lung morphogenesis. *Physiol Rev*. 2007;87(1):219-244.

55. Jawerbaum A, White V. Animal models in diabetes and pregnancy. *Endocr Rev*. 2010;31(5):680-701.
56. Kay TWH, Thomas HE, Harrison LC, Allison J. The Beta Cell in Autoimmune Diabetes: Many Mechanisms and Pathways of Loss. *Trends in Endocrinology & Metabolism*.11(1):11-15.
57. Lambin S, van Bree R, Caluwaerts S, Vercruysse L, Vergote I, Verhaeghe J. Adipose tissue in offspring of Lepr(db/+) mice: early-life environment vs. genotype. *Am J Physiol Endocrinol Metab*. 2007;292(1):E262-271.
58. Treviño-Alanís M, Ventura-Juárez J, Hernández-Piñero J, Nevárez-Garza A, Quintanar-Stephano A, González-Piña A. Delayed Lung Maturation of Foetus of Diabetic Mother Rats Develop with a Diminish, but Without Changes in the Proportion of Type I and II Pneumocytes, and Decreased Expression of Protein D-Associated Surfactant Factor. *Anatomia, Histologia, Embryologia*. 2009;38(3):169-176.
59. Lawrence S, Warshaw J, Nielsen HC. Delayed Lung Maturation in the Macrosomic Offspring of Genetically Determined Diabetic (db/+) Mice1. *Pediatr Res*. 1989;25(2):173-179.
60. Gewolb IH, Rooney SA, Barrett C, Ingleson LD, Light D, Wilson CM, et al. Delayed Pulmonary Maturation in the Fetus of the Streptozotocin-Diabetic Rat. *Experimental Lung Research*. 1985;8(2-3):141-151.
61. Mulay S, Philip A, Solomon S. Influence of maternal diabetes on fetal rat development: alteration of insulin receptors in fetal liver and lung. *J Endocrinol*. 1983;98(3):401-410.
62. Rieutort M, Farrell PM, Engle MJ, Pignol B, Bourbon JR. Changes in Surfactant Phospholipids in Fetal Rat Lungs from Normal and Diabetic Pregnancies. *Pediatr Res*. 1986;20(7):650-654.
63. Singh M, Feigelson M. Effects of maternal diabetes on the levels, synthetic rates and activities of synthetic enzymes of surface-active phospholipids in perinatal rat lung. *Biochim Biophys Acta*. 1983;753(1):53-59.
64. Nijjar MS, Khangura BS, Juravsky LI. The effect of maternal diabetes on the synthesis and secretion of phosphatidylcholine in fetal and maternal rat lungs in vitro. *Diabetologia*. 1984;27(2):219-224.
65. Moglia BB, Phelps DS. Changes in surfactant protein A mRNA levels in a rat model of insulin-treated diabetic pregnancy. *Pediatr Res*. 1996;39(2):241-247.
66. Radaelli T, Varastehpour A, Catalano P, Hauguel-de Mouzon S. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. *Diabetes*. 2003;52(12):2951-2958.
67. Piddington R, Joyce J, Dhanasekaran P, Baker L. Diabetes mellitus affects prostaglandin E2 levels in mouse embryos during neurulation. *Diabetologia*. 1996;39(8):915-920.
68. Tsai MY, Schallinger LE, Josephson MW, Brown DM. Disturbance of pulmonary prostaglandin metabolism in fetuses of alloxan-diabetic rabbits. *Biochim Biophys Acta*. 1982;712(2):395-399.
69. Braman SS. The global burden of asthma. *Chest*. 2006;130(1 Suppl):4S-12S.