MATERNAL DIABETES AS AN EARLY LIFE RISK FACTOR FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE

By

SRIYANI RANATUNGA

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Department of Physiology and Pathophysiology
Rady Faculty of Health Sciences
University of Manitoba
Winnipeg, Manitoba, Canada

Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is Canada's **fifth leading cause of death** and is primarily caused by smoking. However, only <20% of smokers develop COPD, suggesting that other factors are important. Early-life exposures may also increase COPD risk by impairing lung development and altering response to cigarette smoke (CS). Maternal diabetes (MD) increases the risk for premature birth and childhood asthma, which are risk factors for COPD. However, the direct impact of MD on offspring susceptibility towards CS and COPD remains unexplored. I hypothesize that exposure to MD worsens CS induced lung damage in offspring.

Methods: Six-week-old C57BL/6NJ female mice were fed with a high-fat diet (HFD-45% kcal) to induce diabetes-like features or low-fat (LFD-10% kcal) control-diet for 6-weeks, and throughout pregnancy and weaning. Weaned offspring were fed standard research chow-diet until 8-weeks of age, at which point they were exposed to CS/Room air for 50 mins, twice/day, for four days. Lung function and inflammation were assessed on day five. Lung tissues were collected for DNA methylation and gene expression analysis. Data were analyzed in Prism GraphPad using two-way ANOVA, significance set at $p<0.05$.

Results: Offspring from diabetic pregnancy were significantly heavier at 3-weeks than controls. At 8-weeks the body weight difference maintained only in males. CS exposure significantly increased total lung resistance (14%) and airway resistance (18%) in HFD male offspring compared to control. HFD influenced CS induced immune cell infiltration in a sex-specific manner. Female HFD-CS offspring had significantly decreased neutrophils, $CD4^+$ T-cells and NK cells compared to control-smokers. Male HFD-CS offspring had significantly elevated B-cells and NKT cells compared to HFD-control. At baseline, HFD female offspring had elevated Eotaxin and MIG but reduced IL-1α. CS induced cytokine changes in control animals, which were sex specific, were blunted in HFD exposed offspring. Additionally, I observed changes in gene abundance (*Igf*, *Igfbp3* and *Ephx1*), but without specific changes in DNA methylation in smoking individuals from diabetic pregnancy.

Conclusion: MD alters offspring susceptibility towards CS-induced lung dysfunction and inflammation, in a sex-specific manner. MD may modulate response to cigarettes in early adulthood, suggesting a propensity to future COPD.

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Dedication

To

My beloved family

&

“All the fantastic research personalities,

those who need this,

and love this to inspire….”

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List of Abbreviations

AAT	Alpha-1 anti-trypsin
Ahr	Aryl hydrocarbon receptor
Ahrr	Aryl hydrocarbon receptor repressor
AUC	Area under the curve
BALF	Broncho alveolar lavage fluid
bcDNA	Bisulfite converted DNA
cDNA	Cyclic deoxyribonucleic Acid
CO	Carbon monoxide
COPD	Chronic obstructive pulmonary disease
CS	Cigarette smoke
CYP1A1	Cytochrome P450 1A1
DNA	Deoxyribonucleic acid
DNAm	DNA methylation
DNMTs	DNA methyltransferases
DOHaD	Developmental origins of health and disease
Ephx1	Epoxide hydrolase
ETS	Environmental tobacco smoke
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
G-CSF	Granulocyte colony stimulating factor
GDM	Gestational diabetes
GM-CSF	Granulocyte-macrophage colony stimulating factor
GOLD	Global Initiative for Chronic Obstructive Lung Disease
Gstm1	Glutathione S-Transferase Mu 1
Gstp1	Glutathione S-Transferase Pi 1
GSTs	Glutathione S-Transferases
Gstt1	Glutathione S-Transferase theta 1
HFD	High-fat diet
ICSs	Inhaled corticosteroids
IFNγ	Interferon gamma
IGF	Insulin like growth factor

IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
IGF-2	Insulin-like growth factor 2
IGF-2R	Insulin-like growth factor 2 receptor
IGFBP1	IGF binding protein 1
IGFBP3	IGF binding protein 3
IGFBPs	IGF binding proteins
IL-1	Interleukin-1
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-15	Interleukin-15
IL-17	Interleukin-17
IL-17A	Interleukin-17 A
IL-1α	Interleukin-1 α
IL-1β	Interleukin-1 β
IL-2	Interleukin-2
IL-3	Interleukin-3
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-9	Interleukin-9
IL-10	Interleukin-10
IP	Intraperitoneal
IP-10	Interferon gamma-induced protein 10
IPGTT	Intraperitoneal glucose tolerance test
IR	Insulin receptor
KC	Keratinocyte-derived cytokine
LABA	Long-acting beta agonist
LAMA	Long-acting muscarinic agonists
LFD	Low-fat diet
LIF	Leukemia inhibitory factor

LIX	Lipopolysaccharide-induced CXC chemokine
MCP-1	Monocyte chemoattractant protein-1
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage colony stimulating factor
MD	Maternal diabetes
MIG	Monokine induced by interferon gamma
MIP-1α	Macrophage inflammatory protein-1 α
MIP-1β	Macrophage inflammatory protein-1 β
MIP-2	Macrophage inflammatory protein-2
MMP-12	Matrix metalloproteinase-12
MMP-2	Matrix metalloproteinase-2
MMP-3	Matrix metalloproteinase-3
MMP-8	Matrix metalloproteinase-8
MMPs	Matrix metalloproteinases
MPO	Myeloperoxidase
mRNA	Messenger RNA
NK	Natural Killer
NKT	Natural Killer T
NO	Nitrogen Oxides
PAH	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate-Buffered Saline
PCR	Polymerase chain reaction
proMMP-9	Pro matrix metalloproteinase-9
PTP1B	Protein tyrosine phosphatase B1
PTPN6	Protein tyrosine phosphatase non-receptor type 6 gene
RARB	Retinoic acid receptor-beta
RDS	Respiratory distress syndrome
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-qPCR	Real Time quantitative PCR
SEM	Standard Error of the Mean
SERPINA1	Serpin Family A Member 1
SNPs	Single nucleotide polymorphisms

SO2	Sulfur dioxide
SOX5	SRY-Box Transcription Factor 5
SP-D	Surfactant D
T1DM	Type 1 diabetes
T2DM	Type 2 diabetes
TGF-β 1	Transforming growth factor-β 1
TGF-β 2	Transforming growth factor-β 2
TGF-β 3	Transforming growth factor-β 3
Th-1	T-helper cell 1
Th-17	T-helper cell 17
Th-2	T-helper cell 2
Th-22	T-helper cell 22
TIMPs	Tissue inhibitors of metalloproteinases
TNFα	Tumor necrosis factor α
VEGF	Vascular endothelial growth factor

Productivity

Major Presentations

1. “Prenatal exposure to diabetes increases offspring susceptibility to cigarette smoke induced lung dysfunction”. (2023). Presented at the Manitoba Student Health Research Forum. Winnipeg, MB, CA. **Poster Presentation**

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3. “Prenatal and early-life exposure to maternal diabetes as a risk factor for future COPD (Chronic Obstructive Pulmonary Disease): A study from a mouse model”. (2023). Presented at 19th Annual Child Health Research Day. Winnipeg, MB. **Poster Presentation**

4. “Maternal Diabetes as An Early Life Risk Factor for Future COPD”. (2024). Presented at the Young Investigator’s Meeting (YIM). Winnipeg, MB. **Oral Presentation**

5. “Studying the relationship between prenatal exposure to maternal diabetes and sensitivity to cigarette smoke in adulthood in mice”. (2024). Presented at the American Thoracic Society Conference. San Diego, CA. **Poster Presentation**

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CHAPTER 1: INTRODUCTION

1.1 Chronic Obstructive Pulmonary Disease (COPD)

1.1.1 Prevalence

COPD is the third leading cause of death worldwide and is the second greatest cause of hospital admissions in Canada (Lozano et al., 2012). Globally, 90% of COPD deaths occur in low- and middle-income countries (Halpin et al., 2019; Meghji et al., 2021). Based on the recent epidemiological data, in 2012 more than three million people (accounting 6% from the world population) died of COPD. With the increasing prevalence of smoking in low- and middle-income countries and aging population in high income countries the COPD prevalence is expected to rise over the years, and it is estimated annually over 5.4 million deaths of COPD by 2060 (Lopez et al., 2006). This extreme health burden necessitates new treatment options that can prevent disease exacerbations or, better yet, prevent the disease from developing in the first place. The paradigm of COPD pathogenesis is that it is an adult disease caused by smoking. But however not every person who smokes get COPD. Emerging evidence suggests that the origins of COPD may exist in exposure to early life genetic and environmental risk factors that impair lung function (Martinez, 2016). Understanding how the early life environment can be modified to lower COPD disease risk in adulthood may allow us to prevent the development of COPD rather than relying on therapies that only slow its progression.

1.1.2 Definition

According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is defined as "…. a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, sputum production and/or exacerbations) due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema) that cause persistent, often progressive, airway obstruction." (GOLD 2024). In clinical context, COPD is defined as a reduction in the forced expiratory volume in 1 second (FEV1) as a ratio of the forced vital capacity (FVC). FEV1/FVC ratio declines with severity of COPD. According to GOLD 2024, post bronchodilator FEV1/FVC < 0.7 is mandatory to confirm the diagnosis of COPD. Based on the severity of the patient's airflow limitation following bronchodilator FEV1, there are four progressive stages has defined GOLD I–IV (**Table 1**).

Table 1: Gold grades and severity of airway obstruction (based on post-bronchodilator FEV1). Table adapted from GOLD 2024

COPD patients (FEV1/FVC < 0.7)		
GOLD I	Mild	FEV1≥80% predicted
GOLD II	Moderate	50% ≤ FEV1 < 80% predicted
GOLD III	Severe	30% ≤ FEV1 < 50% predicted
GOLD IV	Very severe	FEV1 < 30% predicted

COPD encompasses two disease conditions emphysema and chronic bronchitis. Emphysema is the destruction of alveoli in the lung. As a result, the alveoli become less elastic. This condition mainly causes shortness of breathing and difficulty in exhaling. Chronic bronchitis involves inflammation and narrowing of airways. Inflammation leads to excess mucus production and causes persistent cough and breathing difficulties due to airway narrowing. Even though these two conditions have distinct characteristics they often occur together in COPD patients, creating a complex condition.

1.1.3 Pathophysiology

As described above COPD is characterized by destruction of alveoli and small airways, leading to progressive loss of lung function and breathing difficulties. COPD is traditionally considered a disease caused by smoking, exposure to other forms of particulate matter, such as indoor and outdoor pollution, increases the risk of COPD (Martinez, 2016). Interestingly, only 15-20% of smokers eventually develop COPD (Lo Tam Loi et al., 2013) and the prevalence of COPD in people who have never smoked is also high, ranging from 25-45% (Salvi & Barnes, 2009), suggesting there are additional factors that may influence risk for COPD. COPD development, progression, and prognosis can be influenced by both genetic factors and environmental factors. Additionally, emerging evidence suggests that early-life exposure to environmental factors can predispose individuals to COPD, by reducing peak lung function, accelerating lung function decline, or increasing sensitivity to lung-damaging factors like cigarette smoke (Deolmi et al., 2023). Paradigm shifting research indicated that deficits in FEV1/FVC are apparent in early life and are associated with future COPD risk (Lovasi et al., 2010) and that early life environmental exposures can cause these early lung function deficits (Svanes et al., 2010). Therefore, the early life environment, during pregnancy and early childhood, could represent a novel, modifiable risk factor in COPD etiology. **In in this thesis, I will discuss the**

relationship between early life environmental factors and future COPD risk, the pathways these risks may act through, and discuss whether prenatal exposure to maternal diabetes is an unexplored risk factor influencing life-long lung health and COPD risk.

1.1.4 Inflammation and Remodelling

Chronic lung inflammation is a hallmark feature of COPD, which leads to progressive and irreversible airflow obstruction, and it contributes to the alveolar destruction. The inflammatory process of COPD involves both innate and adaptive immunity. Once the inflammatory process is established in COPD, it persists even after smoking cessation and get worse over the time (Hogg et al., 2004). The main cells involved in inflammation in COPD includes neutrophils, macrophages and lymphocytes (CD4, CD8 and B lymphocytes). These immune cells infiltrate into the lung tissues and airways and can be detected in the sputum and bronchoalveolar lavage fluid (Hogg & Timens, 2009; King, 2015). Among these inflammatory cells, neutrophils are a marker of COPD severity (Mendy et al., 2018; Singh et al., 2010) and are a particular interest because of their role in remodelling and the irreversible destruction of peripheral lung tissue. Remodelling refers to the structural changes that occurs in the lung, often because of chronic inflammation. These structural changes include thickening of the airways due to increased muscle and connective tissues, narrowing of the airways, and increased mucus production (Hirota & Martin, 2013). This is observed in central airways, distal airways as well as in lung parenchyma (alveoli). In COPD, airway remodelling leads to airway obstruction and parenchymal remodelling contributes to emphysema. The infiltrating immune cells release inflammatory mediators such as cytokines and chemokines, and destructive enzymes, which are associated with progressive destruction of the lung in COPD and has an impact on lung remodelling (Y. Wang et al., 2018).

Neutrophils recruited to the airways of the COPD patient secrete serine proteases such as myeloperoxidase (MPO), Neutrophil elastase, and matrix metalloproteinases (MMPs), specifically MMP1, MMP9, and MMP12 are associated with pathogenesis of emphysema. All these enzymes contribute to alveolar destruction (Bardoel et al., 2014; Hendrix & Kheradmand, 2017; Y. Wang et al., 2017). These MMPs are correlated with measures of lung function and disease progression (Culpitt et al., 2005; Ostridge et al., 2016). Additionally, certain neutrophil derived cytokines including IL-1 and IL-8 are involved in tissue damage and airway remodelling (Baek et al., 2013). In COPD, macrophages are increased in airways, sputum and

BALF and are correlated with alveolar wall destruction and inflammatory responses (Arora et al., 2018). Reactive oxygen species (ROS), MMPs, IL-1β, TNF-α, IL-8, and monocyte chemoattractant protein-1 (MCP-1) are produced by macrophages and are linked to COPD pathogenesis (Barnes, 2014). Therefore, changes to the abundance and activity of innate immune cells are important in the pathogenesis of COPD, and possibly risk for COPD.

Adaptive immunity is also activated in COPD, with the infiltration of B cells and T cells into the airways. T cells ($CD8^+$ and $CD4^+$) have ability to directly destroy the lung tissues by T cell induced lung cytotoxicity or indirectly by macrophage activation. $CD8^+$ cells mainly produce IL-4 and IL-5 and are significantly increased in a COPD lung. IL-4 promotes the release of inflammatory molecules (by activating B cells and mast cells) and IL-5 is important for eosinophilic inflammation, where both involve in airway inflammation and parenchymal damage in COPD (Barnes, 2008; Narendra & Hanania, 2019). Individuals with severe COPD have significantly increased B cell numbers, specifically within lymphoid follicles (congregation of immune cells) (Hogg 2004). B cells have both positive and negative effects in COPD. B cells help to remove pathogens by promoting immune responses and they are also known to produce autoantibodies and proteolytic enzymes. Where these autoantibodies and proteolytic enzymes can target lung tissues and extracellular matrix proteins (Polverino et al., 2016). B cells play a role in the development of COPD, with a particular impact on the emphysema phenotype.

1.1.5 Treatments for COPD

COPD is a significant global health concern and an economic burden. Currently there is no cure for COPD, but it is important to emphasize that this is treatable. Proper management can improve the quality of life and slow the disease progression in a COPD patient. COPD treatment strategies are vary based on the disease severity and symptoms of individuals. Key medications for COPD patients include long-acting beta agonist (LABA), long-acting muscarinic agonists (LAMA), combination of LABA-LAMA, and inhaled corticosteroids (ICSs) (Milne et al., 2024). LABA is a bronchodilator, and it helps to relax the smooth muscle cells around the airways. However, LABA is not suitable for acute symptom relief as they function as long-term control medications. LAMA is another effective treatment for COPD patients as they prevent bronchoconstriction in the lungs. ICSs reduce airway inflammation, and are commonly used to treat asthma, but their use is more limited in COPD. Combination

of these medications offers great efficacy compared to LABAs, LAMAs or ICSs alone. All these treatments help to improve lung function, enhance overall health-related quality of life, and even help to prevent the exacerbations. The current therapies have some limitations and potential side effects as well as these therapies relief symptoms but none of them target the disease line. Therefore, COPD necessitates new treatment options that can prevent disease exacerbations or, better yet, prevent the disease from developing in the first place.

1.2 Lung Development and Lung Function Trajectories

The lung development is a complex and highly regulated process which begins early in gestation and continues beyond birth. Lung morphogenesis occurs in five stages where lung development starts during **embryonic stage** (0-6 weeks of gestation). This stage is marked by formation of trachea, bronchi, and lung buds (Warburton et al., 2010). In **pseudo-glandular stage** (6-17 weeks of gestation) the lung bud give rise to conducting airways including bronchioles and terminal bronchioles. These structures undergo repeated branching, leading to formation of bronchial tree (Maeda et al., 2007). This branching pattern causes the expansion of lung surface area, which promotes gas exchange postnatally. During the **canalicular stage**, which lasts from 16 to 26 weeks of gestation, the lung experiences tremendous expansion and differentiation. During this time the conducting airways further divide to give rise to respiratory bronchioles. Around week 26 of gestation, the **saccular stage** begins. Further branching of respiratory bronchioles occurs to formation of alveolar ducts. At this stage surfactant production starts and this continues until birth. Surfactants are vital in reducing surface tension and preventing alveolar collapse to maintain lung compliance (Nkadi et al., 2009). At the end **alveolar stage**, the terminal ducts mature into alveolar sacs where the gas exchange happens. Alveolarization starts following birth and continues towards early childhood.

The lung continues to grow and develop into early adulthood, resulting in an improvement in lung function overtime. Any changes in lung development linked with lower lung function. Lung function trajectories define a pattern of lung function growth and decline over time that is useful for identifying deficits related to disease. Four primary patterns are typically seen: 1) *normal growth and normal decline*, lung function reaches its peak at age 20 and then gradually deteriorates over time in individual who do not have lung disease; 2) *normal growth and rapid decline*, typically defined by cigarette smoke induced rapid decline in adulthood; 3) *reduced growth (reduced peak) with normal decline*; 4) *reduced growth with rapid decline* (Deolmi et al., 2023). The latter three patterns in Figure 1 are linked to the development of COPD (Allen,

2019; Lange et al., 2015; McGeachie et al., 2016). People with reduced FEV_1 in childhood continue to have a lower FEV_1 in adulthood, further increasing their risk for progression to COPD (Krishnan & Martinez, 2018). Additionally, chronic mucus hypersecretion is associated with excessive FEV_1 decline and increased risk for COPD (Vestbo et al., 1996). It is therefore well understood that alterations in lung function growth, peak, and decline are associated with future COPD risk.

Exposure to environmental stressors during early life, whether in the womb or during childhood, can have detrimental effects on organ development and function, and raise the risk of future diseases, a concept known as the developmental origins of health and disease (DOHaD) (Gillman, 2005). Early-life factors such as cigarette smoke, air pollution, premature birth, bronchopulmonary dysplasia, respiratory illnesses in early life, and childhood asthma have all been linked to declines in maximal lung function in adulthood (Chan et al., 2015; Harju et al., 2016; Gauderman et al., 2004; Kotecha et al., 2013; Sears et al., 2003). These exposures reduce the highest achievable lung function and may increase the likelihood of developing COPD, even with a normal rate of lung function decline (Figure 1). Additionally, these exposures can alter the trajectory of lung function decline, rendering individuals more susceptible to COPD (Belgrave et al., 2018; Bui et al., 2018). Maternal exposure to a high-fat diet results in alveolar simplification, suggesting changes in alveolarization (Mayor et al., 2015a). Another study demonstrated prenatal exposure to nicotine has increased airway branching and total airway length suggesting a decreased pulmonary function (Wongtrakool et al., 2012). However, there is a relative lack of knowledge on how diabetes during pregnancy affects the fetal lung development and the subsequent respiratory health of the children.

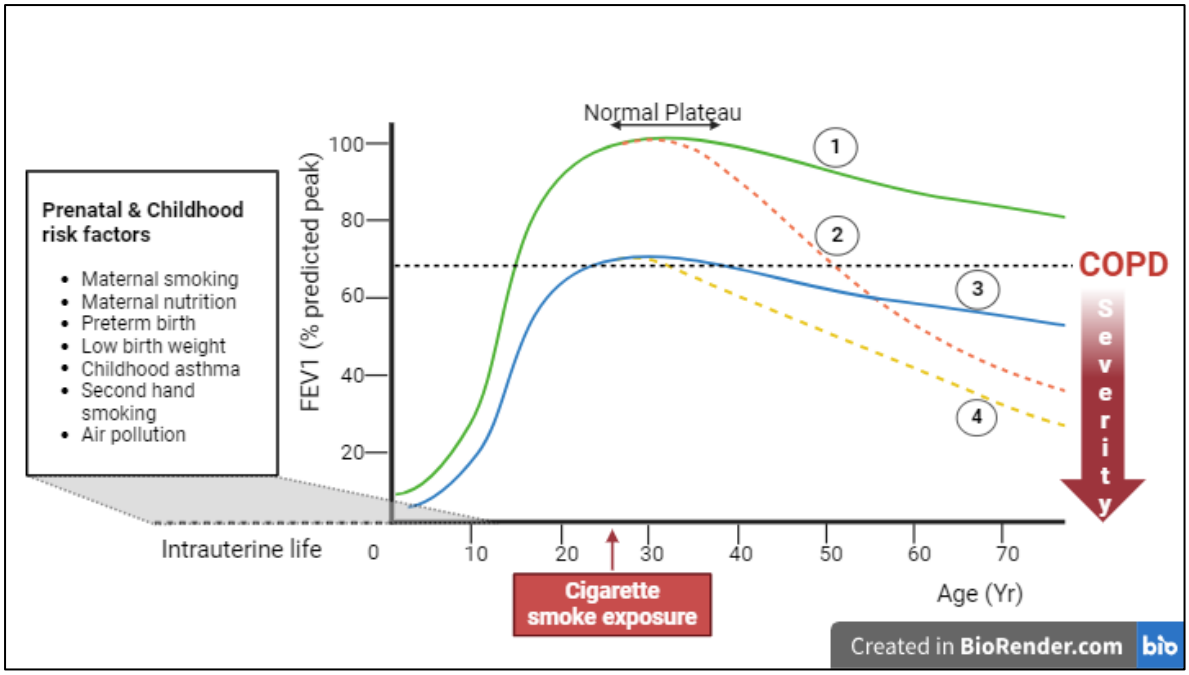


Figure 1: Trajectories of lung function growth and decline that can increase risk for COPD later in life. Early adulthood CS exposure causes a more rapid decline in lung function. Environmental exposures, during pregnancy and early life, could shift lung function to a sub-optimal curve or increase sensitivity to cigarette smoke induced lung function decline, leading to a greater risk for COPD.

1.3 COPD risk factors

COPD is a complex condition resulting from the interplay between genetic factors and environmental influences. An individual's lung function trajectory can be influenced by genetic factors (Simpson et al., 2005; Thyagarajan et al., 2014), environmental exposures like cigarettes or air pollution (Dehmel et al., 2018; Morales et al., 2015), and risk factors in early life (Deolmi et al., 2023).

1.3.1 Genetic Risk Factors

The most well-known genetic risk factor for COPD is alpha-1 anti-trypsin (AAT) deficiency that causes early onset emphysema (Gooptu et al., 2008). AAT is a protein primarily produced in the liver that plays a crucial role in protecting the lungs from damage. AAT deficiency is caused by mutations in *SERPINA1* gene (Nakamura, 2011). Mutations in the *SERPINA1* gene increases the risk of liver and lung emphysema. This is due to insufficient level of circulating AAT to neutralize proteolytic enzymes such as neutrophil elastase in the lungs, resulting the destruction of lung tissues (Deolmi et al., 2023). A recent genetic investigation in a large French-Canadian family with early onset emphysema discovered the second hereditary emphysema in 2019. In this study they found a mutation (Ala455Thr) in the protein tyrosine

phosphatase non-receptor type 6 (*PTPN6*) gene. This mutation in *PTPN6* (also known as SHP-1) reduce the phosphatase activity in the encoded enzyme (SHP-1), which is an important negative regulator of immune processes (Bossé et al., 2019). A family history of COPD constitutes an independent risk factor not only for the development of COPD but also for frequent exacerbations and increased disease severity (X. Huang et al., 2019).

Recent research has focused on finding genetic risk factors for COPD, specifically single nucleotide polymorphisms (SNPs) that are associated with deficits in lung function in healthy people. This follows a new concept in COPD research, the idea that COPD can emerge in a person who has failed to achieve optimal lung function or has a faster than normal decline in lung function regardless of smoking status (Figure 1). This concept of reduced lung growth or early decline in lung function as a factor in COPD pathogenesis allows for the identification of early life factors that influence lung development and COPD risk. Using this definition, a number of SNPs have been identified that are associated with reduced lung growth and an increased risk for COPD (Simpson et al., 2005; Thyagarajan et al., 2014).

Studies using animal models have highlighted the critical roles of certain genes in lung morphogenesis, including retinoic acid receptor-beta (RARB), the transcription factor SOX5, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs). RARB plays a crucial role in the maturation of septation and alveolar formation in murine lungs. Studies have demonstrated that RARB knockout mice exhibit premature septation, underscoring the significance of this receptor in pulmonary development (Carlo Massaro et al., 2000). SOX5, is a susceptibility gene for COPD, which plays a key role in lung morphogenesis during fetal development. SOX5-deficient murine models have demonstrated delayed lung development (Hersh et al., 2011). As described above genetics play an important role in COPD pathogenesis. In addition to genetic factors the environmental exposures can also alter the lung function and COPD risk.

1.3.2 Environmental exposures

1.3.2.1 Tobacco Smoke

The paradigm of COPD pathogenesis is that it is an adult disease caused by smoking. Active or indirect (second hand/third hand) exposures to CS is the largest risk factor in pathogenesis of lung diseases such as COPD (Bartal, 2005; Hogg & Timens, 2009). CS contains noxious

gases and thousands of chemicals including heavy metals, oxidative substances, and carcinogens etc. In utero and early life exposure to CS is associated with a higher risk of asthma and respiratory infections during childhood (Harju et al., 2016b; Jones et al., 2011). Importantly, both asthma and respiratory infections in early childhood are associated with reduced lung function in later life (Chan et al., 2015; Sears et al., 2003). Early life exposure to CS has consistently been associated with decreased lung function in children. Furthermore, children who experience childhood disadvantages, including exposure to parental smoking, have a significantly higher likelihood of developing COPD in adulthood (Svanes et al., 2010). In mouse studies, in utero exposure to CS leads to decreased lung compliance and inspiratory capacity, both of which are hallmark features of COPD (Dehmel et al., 2018; Drummond et al., 2017; Larcombe et al., 2011). Additionally, male mice exposed to maternal smoking show elevated levels of IL-1β and TNF-α in their lungs (Sukjamnong et al., 2017). Prenatal CS exposure worsens lung compliance deterioration in young mice exposed to CS (Drummond et al., 2017). In 2004, one of the European Community Respiratory Health Survey has reported that paternal smoking was associated with increased risk of respiratory symptoms, lower FEV1 and FEV1/FVC ratio in males but not in females, whereas maternal smoking had greater symptoms and poor lung function in the female population (Svanes et al., 2004). Lung function impairment in healthy male adolescents is linked to current exposure to Environmental Tobacco Smoke (ETS), independently of any potential impact from maternal smoking during pregnancy (Rizzi et al., 2004).

Some Research reports show that the prevalence of patients with COPD who have never smoked is also high, meaning that other risk factors also come into play. In addition to parental smoking, Children who experience childhood disadvantages, including parental asthma (maternal/paternal), childhood asthma and pulmonary infections, were significantly associated with lower FEV1 (Svanes et al., 2010). These are defined as ‘childhood disadvantage factors’ and have a greater odd of developing COPD in adulthood.

1.3.2.2 Air pollution

Exposure to air pollutants (other than tobacco smoke) such as sulfur dioxide (SO2), Nitrogen Oxides (NO), Carbon Monoxide (CO) and particulate matters ($PM_{2.5}$, PM_{10}) causes respiratory diseases. Particulate matters trigger oxidative stress which induces epigenetic changes such as DNA methylation, histone modifications and chromatin remodeling (H. J. Kim et al., 2017). These altered gene expressions cause lung inflammations which contribute to the development

of asthma, COPD and other respiratory diseases. Lots of epidemiological studies have shown that exposure to environmental pollutants in-utero and early life leads to lung function impairments in children and adults. Prenatal exposure to $PM_{2.5}$ in late pregnancy has been linked with reduced FEV1 in childhood and DNA hypermethylation in GSTP1 of nasal epithelia, specifically in boys (Lee et al., 2018). In utero exposure to high levels of benzene and NO_2 is associated with lung function reduction in preschool age children (Morales et al., 2015). Sub-chronic exposure to $PM_{2.5}$ and Ozone reduces lung capacity in children at 6-15 years, whereas acute exposure to ozone impairs the small airway function (Chen et al., 2015). Exposure to fine particles in elderly has a more adverse effect on respiratory health. In 2007 Lee et al. observed a decrease of peak expiratory flow with increased exposure levels of fine particles (Lee et al., 2007). Adults who use wood for cooking have significantly impaired lung function compared to the ones who use charcoal as their primary source of fuel (Fullerton et al., 2011). In 2021 another study revealed that the prolonged exposures to wood heating, gas cooking and heating, were associated with higher chances of developing persistent asthma, reduced lung function and percentage reversibility. Additionally, they found evidence suggesting that these effects were influenced by GST genes and ventilation levels (Dai et al., 2021). All the above studies have shown that when individuals are exposed to these pollutants over an extended period, especially in high concentrations, it can have detrimental effects on the respiratory system.

1.3.3 Early life risk factors

1.3.3.1 Childhood Asthma

Asthma is a common chronic respiratory disease marked by airway narrowing and inflammation. Strong evidence indicates that asthma, particularly a history of severe or persistent asthma, increases an individual's risk of COPD later in life. Children with asthma are more likely to develop COPD, an association that is stronger for children with severe asthma, with an odds ratio approaching 32 (Duan et al., 2021; Tai et al., 2014). Childhood asthma is also associated with an accelerated FEV_1 decline in adulthood and asthma-like symptoms are positively correlated with lower pulmonary function at 50 years of age and a doubled risk for COPD (Bisgaard et al., 2021; Tagiyeva et al., 2016). Clinically remittent asthma (controlled asthma, with minimal or no symptoms) in childhood is associated with more airway obstruction at middle age. Specifically, 5.2% of individuals with remitted childhood asthma experience airway obstruction, compared to only 2.2% of healthy individuals (Omori

et al., 2017). It is important to note that while there is a link between childhood asthma and the risk of COPD in adulthood, not every individual with childhood asthma will develop COPD. Children with intermittent asthma did not show an increased risk of developing COPD in adulthood (Tai et al., 2014). However, it is reasonable to conclude that early life factors which increase the risk of asthma, particularly severe asthma, may also be considered potential early life risk factors for COPD.

1.3.3.2 Pre-term birth and low birth weight

Premature birth, defined as the delivery of a baby before 37 weeks of gestational age, can be further categorized as extremely preterm (>28 weeks), very preterm' (28->32 weeks), moderate or late preterm (32->37 weeks of gestational age) (Quinn et al., 2016). Low birth weight can be independent of preterm birth and is defined as a baby weighing less than 2000g or 2100g for females and males respectively. Individuals born prematurely or with low birth weight have impaired lung development, more airway obstruction and lower FEV1 and FVCs in adulthood, with the severity of these changes relating to the severity of prematurity and birth weight. (Doyle et al., 2019; Edwards et al., 2003; Narang et al., 2008). These changes are present by age 8 and persist into adulthood (Kotecha et al., 2012; Lawlor et al., 2005). In women, there is a positive correlation between birth weight and lung function (Lawlor et al., 2005). Furthermore, in women, but not men, preterm birth or low birth weight have elevated risk of asthma and COPD (Broström et al., 2013). Finally, a meta-analysis of 25,000 children links premature birth and low birth weight with an increased risk of childhood asthma (Den Dekker et al., 2016), which is a known risk factor for COPD. Whether the correlation between preterm birth, low birth weight, and COPD is direct, or mediated through changes in asthma risk, is not entirely clear. However, prematurity and low birth weight impact lung function trajectories as highlighted in (**Figure *1***).

1.3.3.3 Surfactant deficiency

Surfactant is crucial for reducing surface tension in the lungs, enhancing compliance, and preventing alveolar collapse during expiration, thus maintaining normal lung function, and preventing tissue damage (Veldhuizen & Haagsman, 2000). People with COPD have lower levels of surfactant and an altered surfactant composition, which may impair its ability to reduce surface tension, contributing to the pathophysiology of the disease (Agudelo et al., 2020a). In COPD, there is a significant reduction in total phospholipids and surfactant lipids in BAL fluid, the deficiency of which correlates with decreased pulmonary function (Lusuardi et al 1992,

Agudelo et al., 2020b). There are established quantitative trait loci in the surfactant D (SP-D) gene that are associated with an elevated risk of developing COPD (Obeidat et al., 2017; Sun et al., 2016). Serum SP-D is negatively correlated with FVC, FEV1 and FEV1/FVC (Lv et al., 2022). Conversely, SP-D is elevated in serum during acute exacerbations and may relate to treatment response (Ju et al., 2012; Liu et al., 2014; Shakoori et al., 2009). The increased serum SP-D during exacerbations may indicate increased leakage at the alveolar interface in the circulation, and the cause-effect relationship between surfactant and COPD pathogenesis still needs clarifying. However, early life exposures that alter alveolar surfactant quality and function are likely to impair lung function and may contribute to COPD risk.

1.4 Maternal Diabetes

Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose levels. Diabetes during pregnancy can be characterized into three distinct groups: pre-existing type 1 diabetes (T1DM), pre-existing type 2 diabetes (T2DM) or gestational diabetes (GDM) (González Corona & Parchem, 2022). Worldwide, there has been a significant increase in the occurrence of all forms of diabetes during pregnancy (Nelson et al., 2024; Stanhope et al., 2021). In Canada, T2DM has increased by 189% (from 2.7 to 7.8 cases per 1,000 deliveries), T1DM has increased by 25% (rising from 2.4 to 3.0 cases per 1,000 deliveries) and GDM has increased by 153% (from 41.2 to 104.3 cases per 1,000 deliveries) over a 15-year study period (Nelson et al., 2024). These statistics highlight the growing concern of diabetes in pregnancy across all types, with T2DM showing the most alarming rate of increase. Diabetes during pregnancy is an important influencer of health, as it is associated with an increased risk of complications affecting both the mother and offspring. Diabetes in pregnancy increases risk of diabetes, impairment of glucose tolerance, obesity, and cardiovascular disease in offspring (Aerts & Van Assche, 2006; Clausen et al., 2009; Dabelea, 2007; West et al., 2011). Importantly, a mounting body of evidence indicates that exposure to diabetes during pregnancy, increases risk for infant and childhood conditions that are risk factors for future COPD, including asthma, premature birth, surfactant deficiency, and impaired lung function.

1.4.1 Maternal Diabetes and Asthma risk

Maternal diabetes has repeatedly been associated with an increased risk of developing asthma in children (Azad et al., 2017). Using data from a Swedish registry, children born to mothers with any type of diabetes during pregnancy had 20% increased odds of developing asthma and

having greater use of asthma medication during childhood (Aspberg et al., 2007, 2010). The association between maternal diabetes and risk of childhood asthma varies by the type of the diabetes. A retrospective birth cohort of over 97 000 births indicates that maternal preexisting type 2 diabetes poses a greater risk for childhood asthma compared to both GDM requiring medication and GDM not requiring medication during pregnancy (Martinez et al., 2020). A racially diverse U.S. pregnancy cohort study confirmed this association, finding that GDM is associated with an increased risk of asthma and wheeze in children (Adgent et al., 2021). Additionally, offspring of mothers with diabetes are more likely to experience persistent wheezing during childhood and develop allergic sensitization, which are both risk factors for future asthma (Rajesh Kumar et al 2009; Rusconi et al., 2007). Maternal diabetes may also magnify the asthma risk associated with prenatal smoke exposure. In children exposed to maternal diabetes, the likelihood of having childhood asthma was 5.7-fold higher when also exposed to prenatal smoke (Azad et al., 2013). Importantly, GDM increases the relative risk of severe, uncontrolled asthma in preschool children, defined as needing short-acting beta-agonist frequently (>7 doses/week), requiring oral corticosteroids, emergency department, or a hospital admission for asthma (Moore et al., 2023). As noted earlier, a history of severe and uncontrolled asthma is linked with an increased risk of COPD in adulthood. However, no longitudinal data exists to determine whether this increased risk for asthma due to maternal diabetes also puts children at a greater risk for COPD. The time between exposure to maternal diabetes, asthma diagnosis, and the development of COPD makes these types of studies in humans challenging.

1.4.2 Maternal Diabetes and Prematurity

Maternal diabetes is associated with an increased risk of preterm birth in offspring (Preda et al., 2023a; Zhang et al., 2012). A population-based cohort study found that premature birth was significantly more common in women with GDM (adjusted OR = 1.29) (Li et al., 2023). Similarly, compared to pregnancies with normal glucose screening, GDM and preexisting diabetic pregnancies more often lead to preterm birth (Hedderson et al., 2003; Köck et al., 2010). Maternal diabetes is also linked with pre-eclampsia, which significantly increases the risk of preterm delivery and low-birth weight, especially in low-income settings (Balaji & Seshiah, 2011). Insulin-treated maternal diabetes is associated with increased risk for preterm births, and in moderately obese mothers with type 2 diabetes there was an increased risk of prematurity (Kong et al., 2019). Finally, mothers with diabetes are more likely to deliver

preterm and their infants may be more likely to develop respiratory distress syndrome, although this is not a universal finding (Grandi et al., 2015; Hedderson et al., 2003; Robert et al 1976). Given the previously established relationship between premature birth, birth weight, and COPD risk, these findings suggest that maternal diabetes may also increase future COPD risk, however, we again lack studies testing this association.

1.4.3 Maternal Diabetes and Surfactant deficiency

Maternal diabetes delays the appearance of the pulmonary maturation marker phosphatidylglycerol, an indicator of surfactant production (Leung-Pineda & Gronowski, 2010; Piper et al., 1998). This association was only seen in poorly controlled diabetic pregnancies, indicating the importance of glycemic control on surfactant production (Piper et al., 1998). Impaired surfactant synthesis and delayed lung maturation can lead to respiratory distress syndrome (RDS) in infants of diabetic mothers (Atar et al., 2021). In animals, maternal diabetes contributes to fetal hyperglycemia and hyperinsulinism, which inhibits surfactant synthesis (Carrapato, 2003; Levine, 1985; Warburton, 1983). Mouse lungs exposed to maternal high-fat diet exhibit an increased cytoplasmic glycogen content, a marker of immaturity, and reduced surfactant protein production (Mayor et al., 2015b). In rats, streptozotocin induced diabetes during pregnancy, which mimics beta cell loss in type-1 diabetes, reduces lung compliance and surfactant production in offspring compared to non-diabetic controls (Baack et al., 2016). As noted previously, reduced surfactant levels are associated with an increased risk for COPD, suggesting that maternal diabetes induced surfactant deficiencies could lead to COPD, although this hypothesis has not been tested (Obeidat et al., 2017).

1.4.4 Maternal Diabetes and Lung Function

A common feature across all chronic respiratory disease is lung dysfunction measured by spirometry, and poor lung function early in life is linked with future COPD risk (Svanes et al., 2010). Therefore, maternal diabetes induced lung dysfunction in offspring could augment COPD risk. To my knowledge there is only one study looked at the effect of maternal glycemia on child lung function using spirometry in humans. In this study they demonstrated maternal GDM is linked with lung function impairments in children specifically with reduced FEV1, FVC and expiratory flow (M. Yang et al., 2024). Several studies have explored the impact of diabetes in adults on lung function, which may provide guidance into understanding the impact of prenatal diabetes on offspring lung function. A diagnosis of diabetes, including type-1

diabetes, is associated with impairments in lung function assessed by spirometry (Innocentia et al., 1994; Lawlor et al., 2004; Walter et al., 2003). In a long-term study tracking lung function in adults over a 16-year follow-up period, adults with diabetes and prediabetes had lower lung function at baseline and experienced a more rapid decline in lung function compared to those without diabetes (Choi et al., 2024). Impaired glucose regulation in individuals without a diagnosis of diabetes is also associated with impaired lung function, specifically, reduced FEV1 and FVC (Lange et al., 1989; McKeever et al., 2005). Most studies on the effect of maternal diabetes on offspring rely on surrogate measures like wheeze, asthma diagnosis or respiratory symptoms to evaluate the impact of maternal diabetes on offspring lung function. Therefore, there is a need for more research directly assessing how maternal diabetes influences lung function using direct measures including spirometry and oscillometry to understand the link between maternal diabetes and future lung disease, including COPD.

In mice, offspring born to high fat diet fed dams have significantly higher lung resistance and lower lung compliance at baseline compared to control low-fat fed dams (Griffiths et al., 2016). In a model specifically testing the effects of maternal diabetes, there are important sex related variations in lung function deficits. Specifically, male offspring exposed to GDM have higher lung compliance and increased airspace size, key factors in COPD pathophysiology, while female offspring showed higher methacholine reactivity and elevated metalloproteases (Pascoe et al., 2022). In a model of type-1 diabetes and high-fat diet, offspring from diabetic pregnancy's having smaller lungs, less surfactant, and altered lung function (Baack et al., 2016). Maternal high-fat diet also reduces lung vascularization, coupled with a reduction in vascular endothelial growth factor (VEGF) receptor 2 and NFκB mediated inflammation, mediated through protein tyrosine phosphatase B1 (PTP1B) (Heyob et al., 2019). Finally, maternal obesity impairs lung function, and increases bronchial and vascular smooth muscle abundance, mediated through IL-6 (Selle et al., 2022). IL-6 and smooth muscle remodelling are key features of chronic lung diseases, including asthma and COPD. Cumulatively, the animal models suggest that maternal diabetes may be an unexplored risk factor for future COPD, mediated through alterations in lung function trajectory.

1.5 Epigenetic Modifications

Epigenetic mechanisms play a crucial role in DOHaD theory. As explained earlier, DOHaD theory proposes that environmental factors during critical developmental periods can shape an

individual's lifelong health trajectory. There are three main classes of epigenetic modifications: DNA methylation (DNAm), histone modifications and non-coding RNA. DNA methylation is the addition of methyl groups to DNA, usually at CpG sites. When this occurs in promoter regions, it usually represses gene expression. Histone modifications are the changes in chromatin structure that impact gene accessibility and gene expression. These changes include acetylation, methylation, and phosphorylation. Non-coding RNAs are RNA molecules that influence gene expression through a variety of processes, such as transcriptional and post-transcriptional regulation, but they do not encode proteins (Yamada & Chong, 2017).

These epigenetic modifications investigate heritable changes in gene expression and persist through cell divisions and can influence cellular function and phenotype without changing the DNA sequence itself. Importantly, environmental exposures (Tobacco smoking, air pollution etc.), diet, aging, and various disease conditions can modulate these epigenetic marks (Onuzulu et al., 2023a; Yang & Schwartz, 2011). The DOHaD theory suggests that early-life experiences can trigger lasting epigenetic changes, potentially influencing disease susceptibility and overall well-being throughout an individual's lifespan. In this thesis I am mainly focus on DNA methylation.

1.5.1 DNA methylation

DNA methylation is the most common and well-studied epigenetic modification in the field of molecular biology. As previously described, this mechanism involves the addition of a methyl group to the DNA molecule, specifically to the 5^{th} carbon of the cytosine ring, which results 5-methyl cytosine. This process of DNA methylation is catalyzed by a group of enzymes known as DNA methyltransferases (DNMTs; DNMT1, DNMT2, DNMT3A and DNMT3B). In mammals DNA methylation primarily occurs at CpG sites, where cytosine is followed by guanine. These sites cluster in CpG islands near gene promoters. Methylation of CpG islands typically suppresses gene expression by impeding transcription factor binding, while unmethylated islands allow genes to remain active. In addition to regulation of gene expression, DNA methylation contributes to genomic imprinting, embryonic development, X-chromosome inactivation, and maintaining chromosome stability (Liyanage et al., 2014; Olynik & Rastegar, 2012).

Previous research has shown that in utero and early life exposures such as smoking, air pollution and diabetic pregnancies etc. can have an impact on DNA methylation and can shape an individual's health trajectory. For an example, in utero exposure to maternal high-fat diet induces changes in DNA methylation patterns in the offspring's brain and liver tissues (Vucetic et al., 2012). Another recent study that found variations in DNA methylation in the cord blood and placenta from humans exposed to GDM (Ruchat et al., 2013). More extensive methylation changes were observed in placenta compared to cord blood in humans. In this thesis I am focusing on DNAm of set of genes involve in two different pathways IGF axis and PAH metabolism pathway, which plays an important role in lung growth and repair, and detoxification of polycyclic aromatic hydrocarbons in CS.

1.5.1.1 IGF Axis

The Insulin like growth factor (Igf) signalling pathway consists of three primary ligands: insulin-like growth factor 1 (Igf-1), insulin-like growth factor 2 (Igf-2), and insulin. These ligands interact with three distinct receptors: the Igf-1 receptor (Igf-1r), the Igf-2 receptor (Igf-2r), and the insulin receptor (Ir). Additionally, the pathway includes a family of six Igf binding proteins (Igfbps 1-6) (Z. Wang et al., 2018). Igf ligands transported bound to Igfbps and play crucial roles in modulating the availability and activity of the Igf ligands.

Igf1 growth hormone axis plays a crucial role in cardiovascular disease, diabetes, malignancy and cognitive function (Green et al., 2014). In addition to that there are some evidence that explain the role of Igf1 axis in relation to lung development and its contribution to cellular repair processes in response to lung injury or disease (Z. Wang et al., 2018). Previous research has shown that there is a positive linear relation between the level of Igf1 and Igfbp3 with FEV1 and FVC in adulthood, the low abundance of Igf1 or Igfbp3 are associated with reductions in lung function (Gläser et al., 2009; Green et al., 2014). The reduced lung function is a major risk factor in development of COPD. Patients with acute exacerbation of COPD, has low serum Igf1 level (Kythreotis et al., 2009). Moreover, Igfbp3 abundance is significantly reduced in smokers, but not in COPD patients (Matsumura & Ito, 2020). At 30 days of age, female offspring from mothers exposed to cigarette smoke had lower Igf1 and Igf1r mRNA levels. Furthermore, they found a sex specific differences in methylation profile in Igf1r promoter region (Meyer et al., 2017). Another study found a reduction of Igf1r and IR mRNA abundance in hippocampus of rat offspring from diabetic pregnancy at 7 days of age (Hami et

al., 2013). Another study found that the maternal diabetes impairs Igf1r and Ir expression in rabbit blastocysts (Ramin et al., 2010). Based on previous research, Igf signaling is crucial for lung development and differentiation, and its dysregulation is associated with COPD, underscoring the importance of Igf1 axis in maintaining lung health and highlighting its potential as a therapeutic target for managing COPD and its complications.

1.5.1.2 Polycyclic Aromatic Hydrocarbon Metabolism Pathway (PAH)

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds made up of carbon and hydrogen atoms, characterized by the presence of a minimum of two fused benzene rings (Buculei et al., 2022). PAH mainly from cigarette smoke and air pollution causes detrimental effects on in the lungs. PAH binds to aryl hydrocarbon receptor (*Ahr*) and trigger the transcription of enzymes which involve in PAH metabolism pathway and induce its own metabolism (Goedtke et al., 2021). Some of these enzymes involve in PAH metabolism pathway are glutathione-S-transferases (*Gstm1*, *Gstt1*, *Gstp1*), cytochrome P450 1A1 (*Cyp1a1*), and epoxide hydrolase (*Ephx1*). These enzymes are important in detoxification of PAH in CS. Some research has shown that these enzymes have been associated with COPD pathogenesis.

Early-life exposures can reprogram the epigenetic landscape, making individuals more susceptible to cigarette smoke induced modifications later in life. The enzymes involved in PAH metabolism typically increase in response to CS, however in COPD, their expression is decreased, and DNA methylation is increased (Vucic et al., 2014). Prenatal CS exposure increased *Cyp1a1* methylation in children and the degree of methylation positively correlates with the smoking intensity (Wiklund et al., 2019). Furthermore, early life CS exposure leads to a greater lung function impairments and DNA methylation changes to the *Cyp1a1* gene upon re-exposure to cigarettes in adulthood (Onuzulu et al., 2023). Interestingly, *Cyp1a1* activity is reduced in mice on a high-fat diet, indicating potential interaction between metabolic factors and PAH metabolism (Sadler et al., 2018). Activation of Ahr pathway, upregulates Cyp1a1 enzyme activity, where this activation increases the production of pro-inflammatory cytokines, such as IL-8 and matrix metalloproteinases (MMPs), which are important in the pathophysiology of COPD (Churg et al., 2012; Di Stefano et al., 2004). Studies have shown significant downregulation of *Gstp1* and *Gstm1* mRNA levels in the peripheral lung tissue of COPD patients compared to those non-COPD. This reduced mRNA expression of these genes correlated with the degree of airflow limitation, suggesting a potential role in disease

progression (Tomaki et al., 2007). Enzymes involved in PAH metabolism, which are in the Ahr signalling pathway, have previously been associated with COPD and display increased methylation in lung tissue of patients with COPD (Ding et al., 2019; Di Wang et al., 2015). Changes in the DNA methylation, and expression, of these genes may relate to mechanisms increasing propensity to CS induced damage by reducing optimal lung function and increasing sensitivity to PAH in CS (PAH metabolism).

1.6. Thesis Overview

1.6.1 Gap of Knowledge

Evidence from human and animal studies indicates that exposure to maternal diabetes increases the risk for childhood asthma, prematurity, surfactant deficiency, and lung dysfunction, which are known risk factors for future COPD (Dumas et al., 2022; Carrapato, 2003; Pascoe et al., 2022; Preda et al., 2023) (**Figure *2***). However, it is unknown whether this link is mediated through established COPD risk factors, or if there is a direct link between exposure to maternal diabetes *in utero* and COPD development later in their life. Potential direct effects of maternal diabetes on COPD development are through alteration of lung development and airway dysanapsis (Ross et al., 2024) or through the activation of COPD pathogenic pathways that drive tissue destruction, such as protease antiprotease imbalance (Lomas, 2016). Alternatively, maternal diabetes may synergise the impact of environmental factors that are known to drive COPD pathogenesis, such as cigarette smoke. But only a small percentage of smokers develop COPD. There are additional factors, genetic or environmental, that can modulate the persons sensitivity to COPD following cigarette smoke exposure.

Few numbers of human and animal studies have explored the synergistic effect of prenatal smoking and active smoking in adulthood on lung function and potential increased susceptibility towards COPD. A prospective human study from birth to age 26, found a synergistic effect between parental and active smoking in adulthood on early lung function impairment. At age 26, individuals exposed to both parental and active smoking had significantly reduced pre-bronchodilator FEV1/FVC levels, with a decrease ranging from 0.9-4.8% compared to those without such exposures. No significant differences in lung function were observed at earlier ages (11, 16, or 22) based on parental or active smoking exposure (Guerra et al., 2013). Moreover, this dual exposure to prenatal and adulthood smoking was associated with a greater risk of hospitalizations/death from COPD (Magnus et al., 2018).

Another study found that the combination exposure to maternal smoking during pregnancy and personal smoking in adulthood is associated with more pronounced airflow limitations in offspring compared to the effects comes from either exposure alone (Upton et al., 2004).

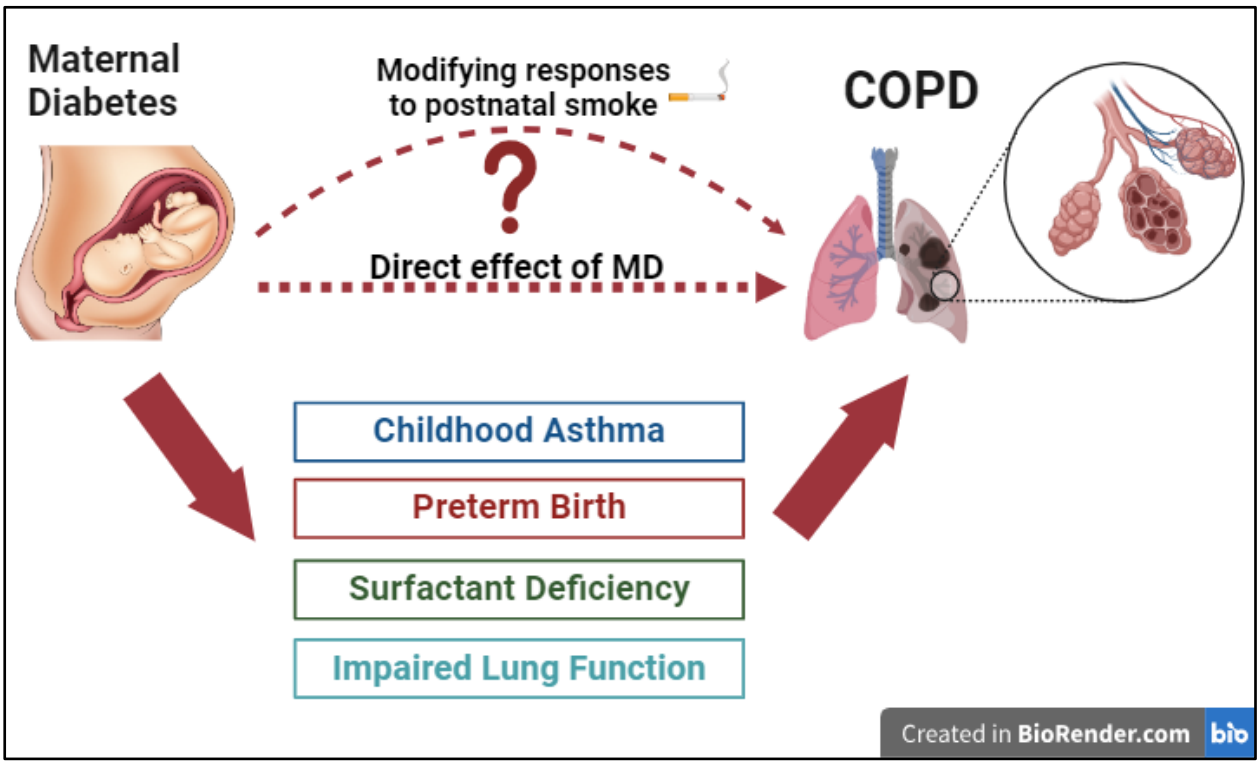


Figure 2: Early life risk factors for COPD. The link between exposure to maternal diabetes in utero and early life, and the risk of developing COPD in offspring later in their life. MD, Maternal diabetes; COPD, Chronic obstructive pulmonary disease

A few animal studies also have explored the impact of environmental exposures on cigarette smoke sensitivity specifically, how prenatal smoking impacts the damage caused by postnatal smoking in offspring. Prenatal cigarette smoke exposure significantly lowers lung compliance and elevates lung elastance compared to their controls, an effect that is worsened with the addition of adolescent CS exposure (Drummond et al., 2017). The combined exposure group (prenatal/adolescent) had the lowest compliance value and the highest elastance compared to prenatal only and control exposure groups, suggesting an ability of prenatal smoking to augment the damage of smoking in adulthood. Another study showed that early life cigarette smoke exposure primes the lungs, leading to greater lung function impairments and DNA methylation changes to the *Cyp1a1* gene upon re-exposure to cigarettes in adulthood (Onuzulu et al., 2023a). Early-life cigarette smoke exposure can reprogram the epigenetic landscape, making individuals more susceptible to cigarette smoke induced modifications when they are

re-exposed later in life. From these studies, we conclude that prenatal and early life factors can increase the sensitivity to cigarette smoke in adulthood, potentially making individuals more susceptible to future COPD.

Thus far, there has been no research investigating whether exposure to maternal diabetes can also directly influence offspring responses to cigarette smoke induced lung damage, a surrogate for COPD risk in future. **Additionally, there are no studies have explored how MD affects DNA methylation and gene expression of Igf axis and PAH metabolizing enzymes in offspring and their sensitivity to the negative effects of CS**.

1.6.2 Hypothesis

I hypothesis that exposure to MD increases offspring sensitivity to CS induced lung dysfunction and inflammation. In these lungs, genes relating to lung growth and repair, and PAH metabolism pathway will have altered gene expressions and DNA methylations.

1.6.3 Objectives and Specific Aims

Objective 1: To determine the effect of exposure to MD on offspring sensitivity to CS induced airway inflammation and lung dysfunction.

> ***Aim 1***: To understand whether exposure to MD increase offspring sensitivity to CS induced lung dysfunction?
> ***Aim 2***: To understand whether exposure to MD increase offspring sensitivity to CS induced lung inflammation?

Objective 2: To understand how exposure to MD affects the Igf1 axis and PAH metabolism enzymes in offspring lung health.

> ***Aim 1*:** To understand how prenatal exposure to MD alter the DNA methylation of genes in Igf1 signalling and PAH metabolism in offspring?
> ***Aim 2***: To determine how prenatal exposure to MD alter gene expression of Igf1 and PAH metabolism genes in response to CS?

CHAPTER 2: MATERIALS AND METHODS

2.1 Study animals

All animal experiments were performed according to standard operating procedures and guidelines approved by the University of Manitoba Animal Ethics Committee (No. B2021-011). C57BL/6NJ female mice (5-week-old) were purchased from Jakson laboratory and were housed in a controlled pathogen free environment (12 hours dark/light cycle). Mice were randomly assigned to cages with a maximum of four mice per cage and acclimatized for one week before the start of the experiment. Mice were fed with a special diet as described below. Offspring refers to mice generated by in house breeding from female mice exposed to special diets.

2.2 Maternal diet exposure

Six-week-old female C57BL/6NJ mice were subjected to a high-fat diet (45% kcal) for a period of 6-weeks to induce weight gain and glucose intolerance. During the same period, control female mice were given a low-fat diet (10% kcal) (**Figure 3**) (Pascoe et al., 2022). The contents of these diets are shown in **Table *2***. Food consumption and weight gain were monitored weekly. The low-fat diet contained a fat content comparable to the standard research chow diet commonly provided to the mice in our facility, which has a fat content of 14%. Male mice used for breeding were given the standard research chow diet, except when they were paired with females for breeding purposes.

Table 2: Composition of High Fat Diet and Low-Fat Diet used to feed mice.

	High Fat Diet		**Low Fat Diet**	
	gm%	kcal%	gm%	kcal%
Protein	24	20	19.2	20
carbohydrate	41	35	67.3	70
Fat	24	**45**	4.3	**10**
Ingredient	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	72.8	291	315	1260
Maltodextrin 10	100	400	35	140
Sucrose	172.8	691	350	1400

Cellulose, BW200	50	0	50	0
Soybean Oil	25	225	25	225
Lard	177.5	1598	20	180
Mineral Mix S10026	10	0	10	0
Dicalcium Phosphate	13	0	13	0
Potassium Citrate, 1 H2O	16.5	0	16.5	0
Calcium Carbonate	5.5	0	5.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0

2.3 Glucose Tolerance Test

At the end of six weeks of diet female mice were fasted overnight (18 hrs.) prior to the test date. For each mouse fasting/baseline blood glucose level was measured using blood from tail vein before glucose administration. After baseline measurements, freshly prepared 10% glucose was administered to mice by intraperitoneal (IP) injection and the appropriate glucose dosage was calculated based on the body weight of each mouse (10 µL/g of mouse from 10% glucose solution). After glucose administration, blood glucose levels were measured and recorded for each mouse at different time intervals (10, 20, 30, 60, and 120 minutes) using blood from tail vein. To measure the blood glucose levels, we used Contour® Next blood glucose monitoring system in our lab. Finally, data were analyzed to confirm the glucose intolerance. Once glucose intolerance was confirmed, the mice were bred to produce offspring. A maximum litter size of 6 used in this study and excess pups at birth were randomly chosen to collect tissues for future analysis. The same diet regimen was continued throughout pregnancy and until the offspring were weaned at 3 weeks of age.

2.4 Offspring postnatal cigarette smoke exposure

At three weeks of age, the mice were introduced to a standard research chow and the diet was continued until they reached eight weeks of age. During this period, the mice were not subjected to CS. At 8-10 weeks of age each litter was split into two groups, one group for CS and the other one for control. One group of mice were exposed to CS for 50 minutes twice a day, four days a week. In parallel, control mice were subjected to room air for the same length of time to imitate the stress associated with the exposure (**Figure 3**).

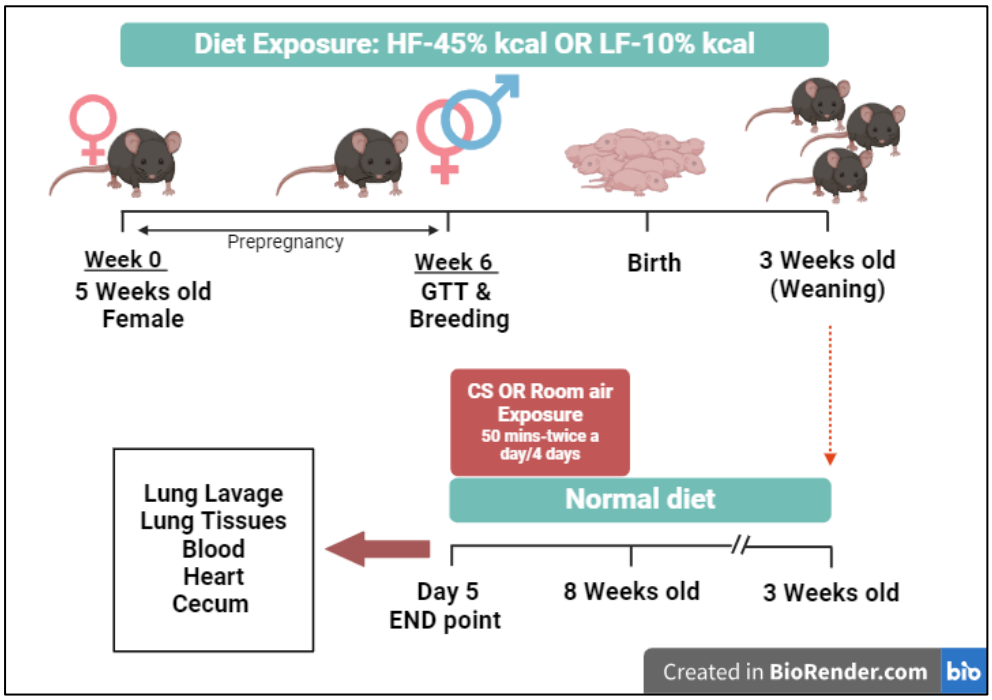


Figure 3: Mouse exposures and sample collection schematic

2.5 Lung function

The mice were anesthetized using sodium pentobarbital (90 mg/kg) administered by intraperitoneal injection. Then the trachea was ventrally opened through the neck and a 20-gauge polyethylene catheter was carefully inserted into the trachea. The catheter connected to the trachea was further connected to the flexiVent small animal ventilator (SCIREQ, Montreal, QC, Canada). The mice received ventilation with a tidal volume of 10 ml/kg of body weight, at a rate of 150 breaths per minute. At baseline Total lung resistance (Rrs), Newtonian resistance (Rn), Tissue damping (G), Tissue elastance (H), were measured. Readings were accepted only if the threshold criterion (coefficient of determination) was > 0.95. For each mouse 12 measurements per parameter were averaged and used for further statistical analysis.

2.6 Sample Collection

2.6.1 Bronchoalveolar Lavage Fluid (BALF)

Following lung function measurements, mouse lungs were washed with Phosphate-Buffered Saline (PBS), 1 mL of PBS gently instilled into the lungs through the tracheal cannula using a syringe and aspirated to collect the lavage fluid. This was repeated twice and the BALF samples were kept on ice. Then samples were centrifuged at 4°C for 10 minutes at 1100 rpm. Cell-free

supernatant was transferred to a new vial and aliquoted to 250-500 µL. These aliquots were stored at -80°C for later cytokine analysis. The cell pellet was used for cell differential assessment.

2.6.2 Tissue samples

Mouse left and right lung lobes were collected separately. The left lung lobes were removed, cut into small pieces using a sterile sharp scissor and placed in a 2mL tube and immediately flash frozen in liquid nitrogen and were stored at -80°C until used for DNA and protein extraction. The right lung lobes were cut out into smaller pieces and transferred into a 2 mL Eppendorf tube containing 1 mL of RNAlater® solution (Invitrogen by Thermo Fisher Scientific) and were kept at 4°C in RNAlater® for 24 h and then moved to -80°C until used for RNA extractions in future.

2.7 Cell counts and Flow cytometry

For quantification of total cell count and differential cells the pellet obtained from BALF was resuspended in 1 mL flow buffer (PBS with 0.5% BSA). The total immune cell count was determined using a hemocytometer.

A portion of BAL cells were used for the immunophenotyping of inflammatory cells by flow cytometer. Cells from the mouse spleen were used to set up gating for compensation. Cells were collected by grinding the tissue with a syringe plunger through a cell scraper sitting on top of a 50 mL tube. Then the screen was flushed with 6 mL of RPMI media and centrifuged for 5 mins at 1200 rpm. The pellet was resuspended in 3 mL of red blood cell lysis ACK buffer (8.02 g of ammonium chloride, 1 g of potassium bicarbonate and 0.03 g of EDTA in 1 L of distilled water) and kept it on ice for 3-4 mins. After red blood cell lysis, the cells were washed with 10 mL of RPMI to neutralize and then centrifuged for 5 mins at 1200 rpm. Then the pellet was resuspended in 10 mL of flow buffer and the cell suspension was filtered using a cell scraper. The number of live cells were counted using TC20™ automated cell counter (Bio-Rad) using trypan blue.

2×10^6 cells from the spleen cell suspension and 1×10^6 cells from the BALF suspension were resuspended separately in 1 mL flow buffer and centrifuged for 5 minutes at 1200 rpm. Then the cell pellet was incubated on ice for 10 minutes after adding Fc blocker (100 µL per tube).

Next the cells were washed with 1 mL of flow buffer and samples were centrifuged. Harvested cells from the BALF were stained with a mixture (0.5µl each antibody/20µl flow buffer/tube) containing following anti-mouse Abs (**Table 3**) and samples used for the compensation were stained with single antibodies (0.5µl antibody/20µl flow buffer/tube) (**Table 3**). After 30 minutes incubation with antibodies mixture, cells were washed with flow buffer and cells were fixed with 2% paraformaldehyde (500µl/tube) for 15 minutes. After that, again cells were washed with 1 mL of flow buffer and cells were harvested by centrifugation. Excess liquid was blotted out using a paper towel and cells were resuspended in 500µL flow buffer and stored at 4°C until use. First the compensation was completed in the machine and then BALF samples labelled with granulocyte and leukocyte panels were acquired using Attune NxT acoustic focusing cytometer (Invitrogen by Thermo Fisher Scientific, Life Technologies Holdings Pte Ltd., Singapore). Finally, data were analysed using FlowJo (Version 10.8.1) software, with gating strategies outlined in **Figure 4** for cell surface markers (**Table 4**). To calculate the absolute cell numbers for each type of cells, the percentage distribution was multiplied by the absolute total cell count in the BALF.

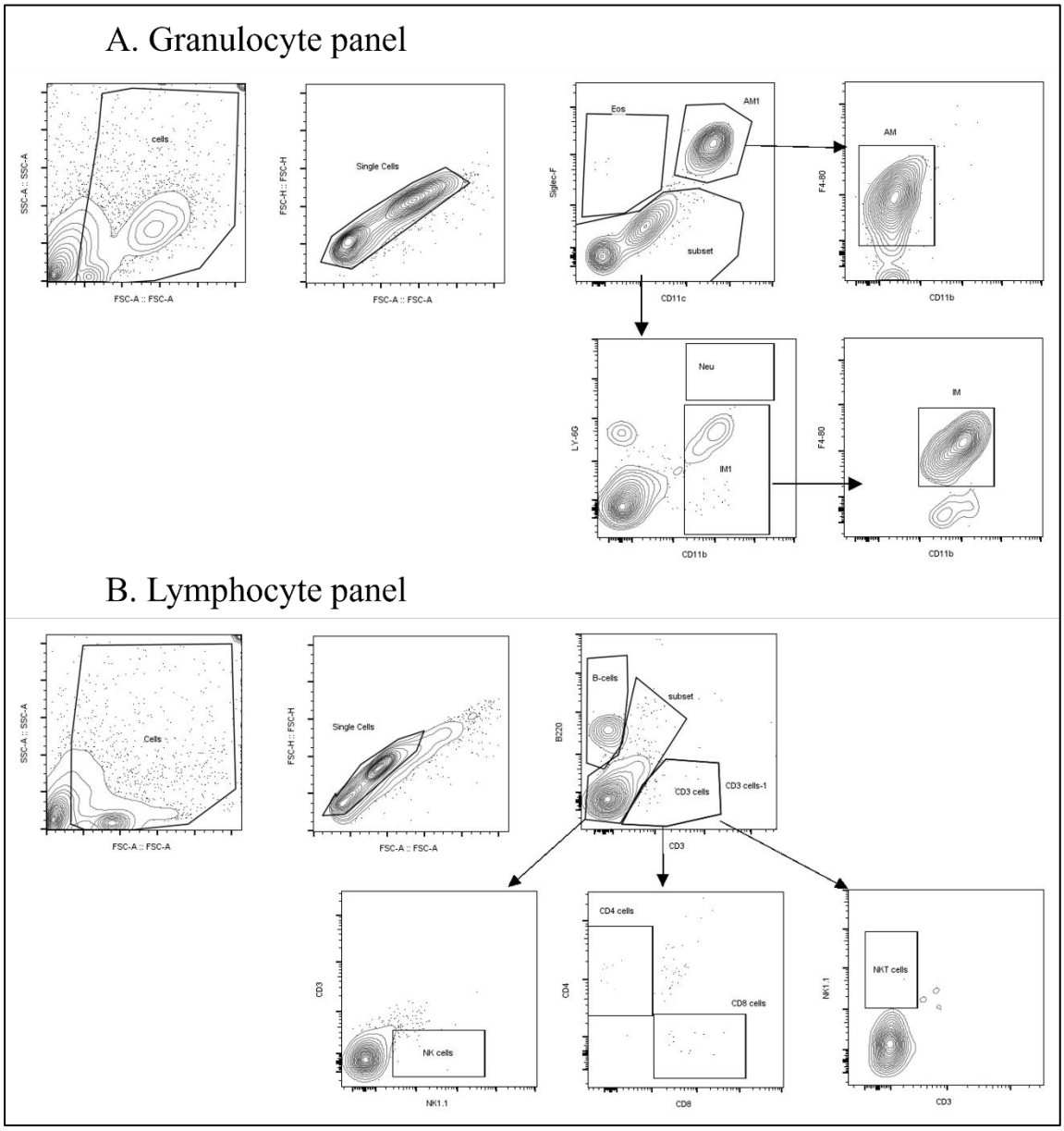


Figure 4: Gating strategy for flow cytometry. Differential immune cells were identified using the gating strategy shown above. (A) granulocyte panel and (B) lymphocyte panel. AM: alveolar macrophages, Eos: eosinophils, IM: interstitial macrophages, Neu: neutrophils

Table 3: Anti-mouse antibodies used to stain BALF cells (for granulocytes and Leukocytes) and spleen cells

Granulocytes Panel	Leukocytes panel	Compensation/Spleen cells
APC anti-mouse Ly6G	APC anti-mouse B220	APC anti-mouse CD4
Percp-Cy5.5 anti-mouse F4/80	Percp-Cy5.5 anti-mouse CD4	Percp-Cy5.5 anti-mouse CD4
FITC anti-mouse CD11c	FITC anti-mouse CD8a	FITC anti-mouse CD4
PE anti-mouse CD170 (Siglec-F)	PE anti-mouse CD3	PE anti-mouse CD4
PE-Cy 7 anti-mouse CD11b	PE-Cy 7 anti-mouse NK1.1	PE-Cy7 anti-mouse CD4
		APC-Cy7 anti-mouse CD4

Table 4: Cell surface markers for the identification of immune cells in granulocyte and leukocyte panels

	Immune Cell Type	Cell surface markers
Granulocyte Panel	Eosinophils (Eos)	$CD11c^{low}$ Siglec-F^{+}
	Neutrophils (Neu)	$CD11c^{low}$ Siglec-F^{-}, $CD11b^{high}$ Ly-$6G^{+}$
	Alveolar Macrophages (AM)	$CD11c^{high}$ Siglec-F^{+}, $CD11b^{int}$ $F4/80^{+}$
	Interstitial Macrophages (IM)	$CD11c^{low}$ Siglec-F^{-}, $CD11b^{high}$ $F4/80^{+}$
Leukocyte Panel	B cells	$B220^{+}$
	CD3 cells	$CD3^{+}$
	CD4 cells	$CD3^{+}$, $CD4^{+}$
	CD8 cells	$CD3^{+}$, $CD8^{+}$
	Natural Killer T cells (NKT)	$CD3^{+}$, $NK1.1^{high}$, $CD3^{+}$
	Natural Killer cells (NK)	$CD3^{low}$ $B220^{-}$, $NK1.1^{+}$

2.8 Cytokine analysis

This study used Luminex xMAP technology for multiplexed quantification of 40 mouse cytokines, chemokines and growth factors.

The multiplexing analysis was performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). Forty markers were simultaneously measured in the samples using Eve Technologies' Mouse Cytokine 32-Plex Discovery Assay®, MMP 5-Plex Discovery Assay®, TFG-β 3-Plex Discovery Assay® (Millipore Sigma, Burlington, Massachusetts, USA) according to the manufacturer's protocol. The 32-plex consisted of Eotaxin, G-CSF, GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, TNFα, and VEGF. Assay sensitivities of these markers range from 0.3 – 30.6 pg/mL for the 32-plex. The 5-plex consisted of MMP-2, MMP-3, MMP-8, proMMP-9, and MMP-12. Assay sensitivities of these markers range from 1.6 – 8.4 pg/mL for the 5-plex. The 3-plex consisted of TGF-β 1, TGF-β 2, and TGF-β 3. Assay sensitivities of these markers range from 2.2 – 6.6 pg/mL for the 3-plex. Individual analyte sensitivity values are available in the Millipore Sigma MILLIPLEX® MAP protocol.

2.9 Pyrosequencing: Measuring DNA methylation of Igf1 axis and PAH metabolism

2.9.1 DNA extraction

Flash frozen left lung was subjected to DNA isolation. Total DNA was extracted from 25 mg of minced lung tissue using PureLink® Genomic DNA Kit according to the manufacturer's instructions as indicated in the user guide. In brief, tissue was homogenized in PureLink® genomic digestion buffer and Proteinase K using the TissueLyserII (Qiagen®) for 2x 20s at 15Hz. Then the lysate was centrifuged to remove any cellular debris, and the supernatant was transferred to a new sterile microcentrifuge tube. Then RNAse, PureLink® Genomic binding buffer and 100% ethanol were added to the lysate and processed as described in the protocol. The sample was mixed by vortexing and then transferred to a PureLink® Spin Column in a collection tube to proceed to DNA binding. Then the column was washed twice with the wash buffers to remove any residual contaminants. Finally total DNA was eluted in 100 µL of PureLink® Genomic Elution Buffer. The quantity and quality of the extracted DNA were assessed using a Qubit 4 fluorometer (Invitrogen by Thermo Fisher Scientific) and Nanodrop 2000 Spectrophotometer (Thermo Scientific). The extracted DNA was stored at –20 °C for long term storage.

2.9.2 Bisulfite conversion and Pyrosequencing

Pyrosequencing was used to quantitively estimate methylation levels at CpG sites within the promoter region in total of 11 genes, with the help of Dr. Meaghan Jones lab in the Department of Biochemistry and Medical Genetics. For this study I designed primers targeting the promoter region and the gene sequences of interest were obtained from UCSC Genome Browser. Pyromark Assay Design Software 2.0 (Qiagen) was used to design bisulfite pyrosequencing assays and primers. Primer sequences are listed in **Table *5***. Genomic DNA (200-500 ng) was bisulfite-converted using the EZ DNA Methylation-Gold^{TM} Kit (Zymo Research) as per manufacture's protocol. The eluted bisulfite-converted DNA samples were quantified by Nanodrop 2000 Spectrophotometer (Thermo Scientific) and then diluted down to 15 ng/µl for PCR amplification.

The region of interest was amplified by PCR with the forward and reverse primers (10 µM) using HotStar Taq DNA Polymerase Kit (Qiagen). We made sure that one of the primers,

forward/reverse was biotinylated. PCR was performed in a thermal cycler (Veriti thermal cycler), and each PCR reaction mixture (40 µL) included 4 uL of 10x buffer, 2.4 uL of $MgCl_2$, 0.8 uL of dNTPs (10mM), 0.8 uL of 10 uM of each Forward and Reverse primers, 0.2 uL of HotStar Taq Polymerase, 30 uL of distilled H2O and 1uL of bcDNA sample (15 ng/uL). The PCR reaction conditions used were as follows: 95°C for 15 minutes, 45 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, then the final extension was at 72°C for 5 minutes. The resulting PCR products were examined by agarose gel electrophoresis using a 1.5% agarose gel (1.5g of agarose in 100 ml 1X TBE buffer) with a 1kb plus DNA ladder (Invitrogen by Thermo Fisher Scientific). The gels were visualized under UV illumination using Chemi-Doc gel imager (Bio-Rad).

Finally, DNA methylation was measured using Qiagen Q48 pyro sequencer. Prior to running all samples at once, CpG assays were validated. For bisulfite-conversion efficiency and quality control, Mouse High Methylated and Low Methylated DNA controls (EpigenDx) were used to prepare a dilution series of 0%, 25%, 50%, 75%, and 100% methylated controls for a standard curve and were included on each assay.

2.10 qPCR: Measuring gene abundance in Igf1 axis and PAH metabolism

2.10.1 RNA extraction

Measured up to 25-30 mg of frozen right lung tissue in RNAlater and tissues were subjected to RNA isolation using PureLink™ RNA Mini Kit according to the manufacturer's instructions as indicated in the user guide. In brief, before the process the lysis buffer was freshly prepared (10 µL 2-mercaptoethanol+1 mL of lysis buffer). Tissues were homogenized in 600 µL of lysis buffer with 2-mercaptoethanol using the TissueLyserII (Qiagen®) for 2× 2 min at 25Hz. Then the homogenate was centrifuged to remove any cellular debris, and the supernatant was transferred to a clean RNase-free tube. Then 1 volume of 70% ethanol was added to the lysate and samples were mixed by vortexing. Samples were transferred to the spin cartridge in a collection tube to proceed to RNA binding. After samples processed the spin cartridge were washed with Wash Buffer I. Then On-column DNase treatment was performed using 80 µL of PureLink™ DNases mixture and again spin cartridge was washed with Wash Buffer I and Wash Buffer II to remove any residual contaminants. Finally, RNA was eluted in 50 µL of RNase-free water. The quantity and quality of the extracted RNA were assessed using a Qubit

(Invitrogen by Thermo Fisher Scientific) and Nanodrop 2000 Spectrophotometer (Thermo Scientific). The extracted RNA was stored at –20 °C for long term storage.

Table 5: Pyrosequencing Primers, their amplicon size and targeted CpG location on promoter region

Gene	CpG location	Primer Sequences	Amplicon size
Cyp1a1	Chr9:57687860	GTGTGTATAATGAAGGGAGGTAGAAT ACAACACAAACCAAAATATCACC GGGTAGTGAGGAATAG	100
Igfbp1	Chr11:7197478	GGGAGAAATAATTGTGGGTATTGTT AATCCAACTCTATCAACAAACTAC AGTAGTTTAGGTTTTTTGTGAG	201
Igfbp3	Chr11:7214484	TGGTTTTTTTAGGGATGGTTTATGT CTAAAATTCTCCAAACCTCAAAATAATC GTTTGGGAAGTTGGT	164
Ir	Chr8:3279734	TTTTGGTTTTTTAGAGGAGTTAGTAGG ACCAAAAAATACAAACTACCTACA TGAGTTTTAGTTTAGTAGTTTGG	134
Ephx1	Chr1:181018214	TGGGGATAAAGTTTGAGGGTA CTCCTCCCTATACTAACCTCTACT ATAGTGAAGTGTGGAAT	194
Gstm1	Chr3:108018659	GGAATTTGTTATTGGGGAGGTT AACCCTATTTATCTCTACCTTAAAATTATT GTTATTGGGGAGGTTT	202
Gstp1	Chr19:4038121	TGGTTTATAGAGTGAATTTTAGGATAGTTA ACTCAACCTAAAACCTACCTATTCATCTAA TGTGAGAATAGGGATAAAT	159
Gstt1	Chr10:75799332	AGTTGAAAGTAGAATGTTTTTAAAGGTATA AAAAACCCACAACATCCTTTACTTCTTA AGGTATATTTATTTTATTTAGTGTG	171
Ahrr	Chr13:74292734	GGATATTATATGGTGGATAGGTTTAGT AACTACCCACACCAAAAACTTCAAAAATTC GGTGGATAGGTTTAGTTTTAA	138
Ahr	Chr12:35535360	GGAGGGTAGGTATTGAGATTATGATT CTACAAAATAAAACCCAATTTCTTCAT GTAGGTATTGAGATTATGATTAGA	149

2.10.2 cDNA synthesis

1 ug of input RNA was converted to cDNA using the iScript Reverse Transcriptase kit. Total reaction volume was kept at 20uL. The thermal cycler reaction conditions used were as follows: 5 min at 25 °C, 20 min at 46 °C, 1 min at 95 °C. At the end cDNA was diluted in TE buffer to

make the final concentration to 10 ng/µL by assuming 100% efficiency in conversion of RNA to cDNA. The generated cDNA was stored at –20 °C until use for the qPCR.

2.10.3 qPCR

Gene sequences of interest were obtained from NCBI Browser for primer design. Primer sequences were generated using IDT Primer Quest Tool. Additionally, NCBI Splign and Oligo Analyzer IDT were used to identify exon-exon boundaries in mRNA and to check for primer dimers respectively. Finally, the specificity of PCR primers was tested using NCBI Primer blast. Primer sequences are listed in **Table 7**. Temperature gradient RT-qPCR was performed to find the optimum annealing temperatures for each primer. Reaction mix was prepared for all qPCR reactions by adding all required components, according to **Table 6** below. RT-qPCRs were performed in duplicates. GAPDH, YWAHZ and UBC were used as reference genes. RT-qPCR was performed using CFX Connect™ Real-Time PCR Detection System (Bio-Rad, USA). The PCR reaction conditions used were as follows: 95°C for 2 minutes, 40 cycles of 95°C for 15 seconds, 60/62.5°C for 30 seconds, then extension 95°C for 10 and 72°C for 30 seconds, then the final extension was at 72°C for 5 minutes. The stability of the reference genes was determined using RefFinder (http://www.heartcure.com.au/reffinder/). The most less stable reference gene was excluded from the analysis. Additionally, the reaction efficiencies were determined from slope of amplification curve using LinRegPCR software.

Table 6: RT-qPCR reaction mix volumes of each component per reaction.

Components	**Volume per 20 µL reaction**	**Final concentration**
Universal SYBR Green Super mix	10 µL	
Forward and Reverse primers	1 µL from each	10 µM
ddH_2O	4 µL	
cDNA	4 µL	5 ng/µL
Total reaction mix volume	20 µL	

Table 7: RT-qPCR primer sequences of genes used for this study and their amplicon size.

Gene	Primer Sequences	Amplicon size
Igf1 (T_a=60°C)	F: TACTTCAACAAGCCCACAGG R: TCTCCAGTCTCCTCAGATCAC	106
Cyp1a1 (T_a=60°C)	F: GATACCCAGCTGACTTCATTCC R: GGATGTGGCCCTTCTCAAAT	144
Igf1r (T_a=62.5°C)	F: CACCCAGAGCATGTACTGTATC R: CCAACTCCGAGGCAATGTTA	182
Igfbp1 (T_a=62.5°C)	F: CGACCTCAAGAAATGGAAGGA R: TCTCACACTGTTTGCTGTGATA	155
Igfbp3 (T_a=62.5°C)	F: GATGCTCCGTGCCACATAATA R: CAGTTCACCACACTCACACA	148
Ir (T_a=62.5°C)	F: ATCCTGGATTCTGTGGAGGA R: GGGCAAACTTTCTGACAATGAC	173
Ephx1 (T_a=62.5°C)	F: GATCAAGGACTTGCACCAGA R: CTGCTTCCTCCAGTCAAACT	142
Gstm1 (T_a=60°C)	F: CACAAGATCACCCAGAGCAA R: GCAATGGAACAGCCACAAAG	175
Gstp1 (T_a=62.5°C)	F: ACCATTGTCTACTTCCCAGTTC R: GCTGCCCATACAGACAAGT	145
Gstt1 (T_a=62.5°C)	F: GAGTGTGGCTATCTTGCTCTAC R: ACAGGGAACATCACCTTATGC	168
Ahrr (T_a=60°C)	F: CACCAGTCTTGCCTTCTGTAA R: CTCCATTGCTCTTTCCTGCTA	171
Ahr (T_a=60°C)	F: CTTCTAAGCGACACAGAGACC R: GGGTGGACTTTAATGCAACATC	168

2.11 Data Analysis

All litter was culled to a maximum of 6 pups just after the birth on day one. Analysis of sex-based differences in the offspring was performed after collection of all the data. Normality of residuals were assessed with D'Agostino-Pearson omnibus (K2) test along with qqplot (Prism GraphPad 10). When residuals were not normally distributed, data was log transformed. When transformation did not yield normally distributed residuals, analysis was performed on ranks using non-parametric tests. Sex-disaggregated analysis was done to explore differences in male and females individually. Here all data were expressed as mean ± standard error of the mean (SEM). All data analysed using two-way ANOVA with Fisher's LSD multiple comparison test (Prism GraphPad 10), with maternal diet and postnatal smoke as independent variables to

assess the impact of these factors on lung function, inflammation, cytokine production, DNA methylation and gene abundance. We used five litters per diet per CS exposure, which gives enough sample numbers for statistical evaluation to compare the mean of each group. A threshold of $p<0.05$ was used to define the statistical significance. Data were collected from at least five mice per sex, per maternal exposure, per adulthood exposure (room air/CS).

CHAPTER 3: RESULTS

3.1 Maternal weight gain and Glucose intolerance

C57BL6/NJ female mice fed HFD experience a significant weight gain from week 3 onwards and glucose intolerance, a characteristic feature of maternal diabetes. Mice exposed to HFD had a 3.95± 0.77 g increase in weight gain compared to the control 1.73 ±0.43 g (**Figure 5**A). After six weeks of diet intervention, to confirm the glucose intolerance, female mice fed with either control or HFD were subjected to overnight fasting and performed intraperitoneal glucose tolerance test (IPGTT). The fasting blood glucose level was significantly increased in HFD-exposed mice (7.82 ± 0.82 mmol/L) indicating impaired fasting glucose compared to their respective controls (5.56 ± 0.82 mmol/L), P=0.02. During IPGTT, HFD-exposed female mice had an increased blood glucose level after 10 min of glucose administration. After 60 mins there was a significant delay to remove glucose from the blood stream (**Figure 5**B). The area under the curve (AUC) of the IPGTT is also elevated, further signifying impaired blood glucose levels (**Figure 5**C).

3.2 Offspring Morphometrics

There was no significant difference in fertility between female mice that were exposed to HFD and control. Five out of eight (62.5%) from both HFD-exposed and control females were pregnant after initial mating. Control dams had a slightly larger litter size (8.00±0.91) than HFD-exposed (5.50±1.55), but all litters were culled to a maximum of 6 pups to eliminate the effect of litter size. Both male and female offspring from HFD-exposed mothers were significantly heavier at three weeks of age (male 14.84 ± 0.74 g; female 13.31 ± 0.52 g) than controls (male 11.51 ± 0.19 g; female 10.77 ± 0.25 g, where P=<0.0001 and P=0.0007 respectively) (**Figure 5**D). At 8 weeks of age the body weight difference was only seen in males (offspring from HFD 25.28 ± 0.42 g; control 23.82 ± 0.54 g, P=0.02) (**Figure 5**E).

Maternal diabetes alters lung structure in male offspring, independent of cigarette smoke exposure. The Pressure-Volume loop data indicates that HFD male offspring had most compliant lungs and higher-pressure volume loop area compared to controls (**Figure 5**F). In male deflation curve the lung volume was significantly higher in HFD offspring at pressure 2.9, 6.8, 10.7 and 14.6 cmH_2O compared with its control group. This elevation in the deflation limb of the PV-Loop suggests changes in the elastance of the lung and gas trapping. There are no differences seen between HFD and control females. Following 4-day CS exposure we have not seen any further differences in PV loops in either male or female offspring from control or HFD diet (**Supplementary Figure 1**). Which means this 4-day acute CS exposure may not

enough to change the mechanics of the lung in terms of elastance and compliance. It also suggests that the changes seen in the PV loop of HFD males might be due to changes in lung development and structure. These changes are not expected to happen after 4 days of CS.

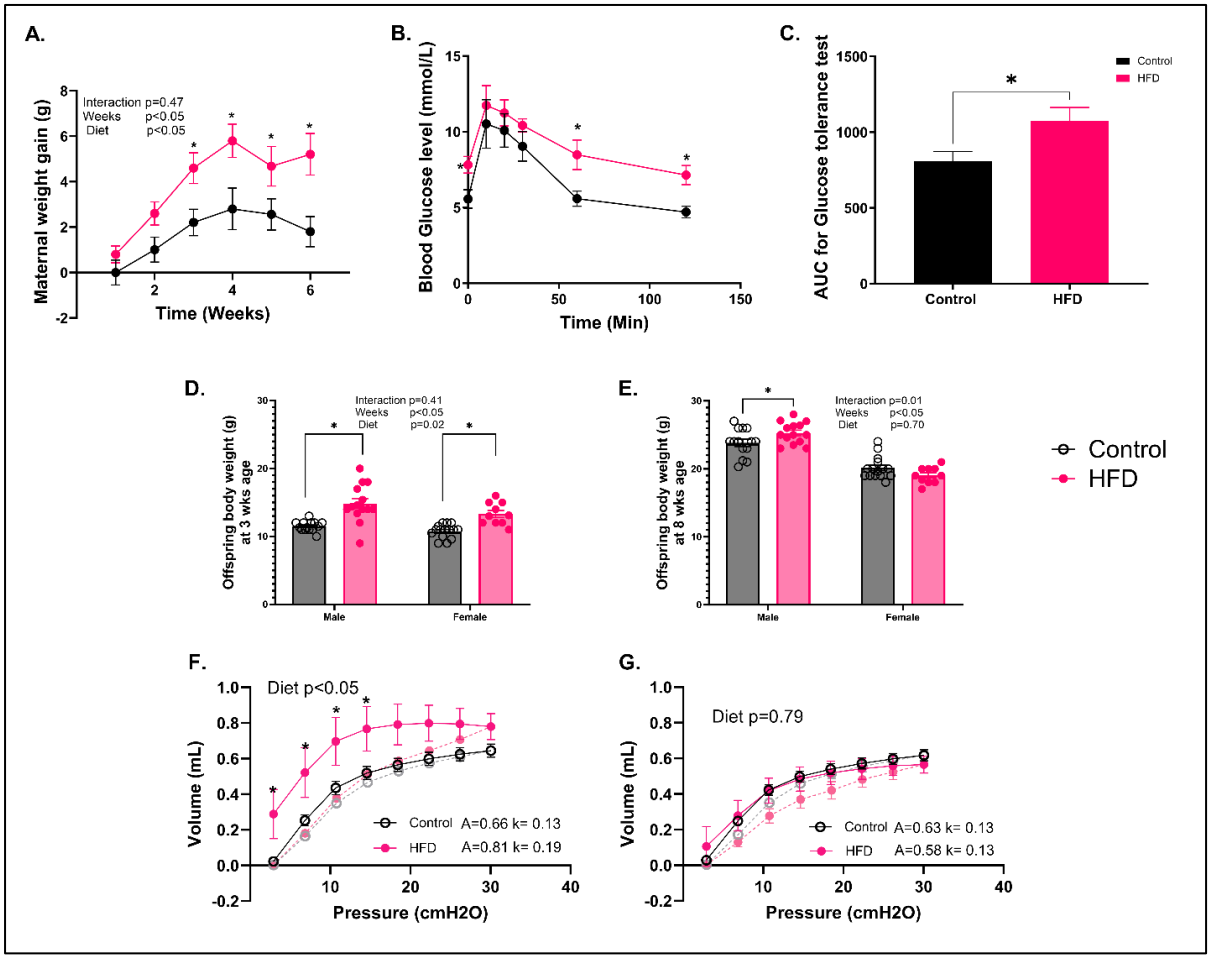


Figure 5: High-fat diet increases maternal body weight and promotes glucose intolerance, and this also associated with morphometric changes in their offspring. **A**: maternal weight gain over six weeks of diet exposure (n=5 for each group), **B**: Change in blood glucose concentration after intraperitoneal injection with glucose (glucose tolerance test), **C**: Area under the curve for glucose tolerance test ($AUC_{glucose}$), **D**: Offspring body weight at 3 weeks of age, **E**: Offspring body weight at 8-10 weeks of age. **F**: PV-loop for male offspring in response to HF diet, **G**: PV-loop for female offspring in response to HF diet. Data on the descending curve are fitted to the Salazar–Knowles' equation: $V = A - Be^{-KP}$, to get the best fitted values for k and A. k defines the curvature of the line, A is an estimate for inspiratory capacity, V and P stand for volume and pressure respectively. *P<0.05 N=5-7 offspring per group. In graph F and G, the dash line shows the ascending curves, and the solid line shows the descending curves.

3.3 Lung function measurements in offspring

Lung function was assessed at 8-10 wks of age for all the mice. There were no significant differences observed in lung function parameters between offspring from control and HFD

exposures in the absence of CS. However, when these mice were exposed to acute 4-day CS, we observed alterations in lung function metrics. Specifically, CS exposure significantly increased total lung resistance by 14% (1.03 ± 0.05 $cmH_2O.s/mL$) (**Figure 6**A) and Newtonian resistance/small airway resistance by 18% (0.57 ± 0.02 $cmH_2O.s/mL$) (**Figure 6**B) in male offspring from HFD-fed mothers compared to HFD controls (0.91 ± 0.05 cmH2O.s/mL and 0.45 ± 0.02 cmH2O.s/mL respectively). The increase in total lung resistance and Newtonian resistance was seen relative to both the HFD-control, and control-CS, indicating an interaction between diet and smoke, where the Newtonian resistance as significant ($p = 0.002$). In HFD females the total lung resistance and Newtonian resistance was numerically increased, but no significant changes were seen in response to CS exposure (**Figure 6**G & H). Furthermore, no significant effects found on other lung function parameters (**Figure 6**) in response to acute CS in either control or HFD offspring.

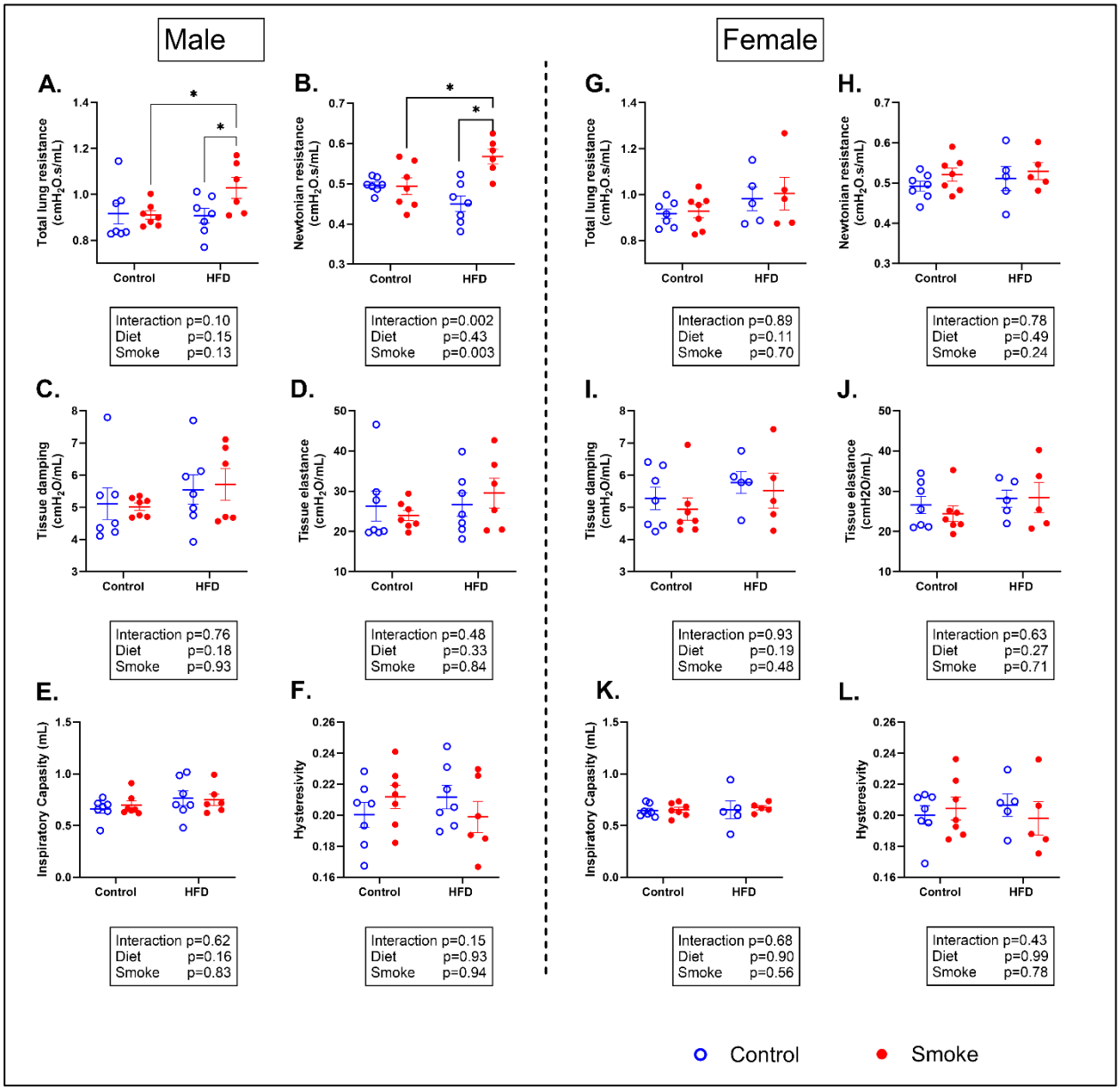


Figure 6: Maternal diabetes exacerbates CS induced lung dysfunction in 8-week-old offspring. Lung function parameters were altered in a sex-specific manner in response to CS exposure. (A, G) Total lung resistance, (B, H) Newtonian resistance/small airway resistance, (C, I) Tissue damping, and (D, J) Tissue elastance, (E, K) Inspiratory capacity, (F, L) Hysteresivity at baseline in male (A-F) and female (G-L) offspring. Lung function was measured 24 hours after the last CS exposure. Each dot represents an individual mouse. Data were analysed in a sex-disaggregated manner, with statistical significance determined using Two-way ANOVA with Fisher's LSD multiple comparison test (*P≤ 0.05). N=5-7 per group.

3.4 Sex Related Differences in Immune Cell Profile

I examined total cell counts and differential cell profiles using BALF samples collected from offspring at 8-10 weeks of age. In the absence of CS, no statistically significant differences were observed in cell counts between offspring from control and high-fat diet (HFD) dams.

However, upon exposure to a 4-day acute CS challenge, the differences became more pronounced. I observed that smoke exposure significantly affects total cell counts (p = 0.03) regardless of diet. Both males and females showed increased total cell numbers due to CS exposure (**Figure 7**A & B). Male HFD-CS offspring had a significantly elevated total immune cell induction compared to their control counterparts (Male HFD-CS had 61430 cells, a 2.14-fold increase from HFD-control, p=0.03). In both control males and females, no significant differences were seen after the CS challenge.

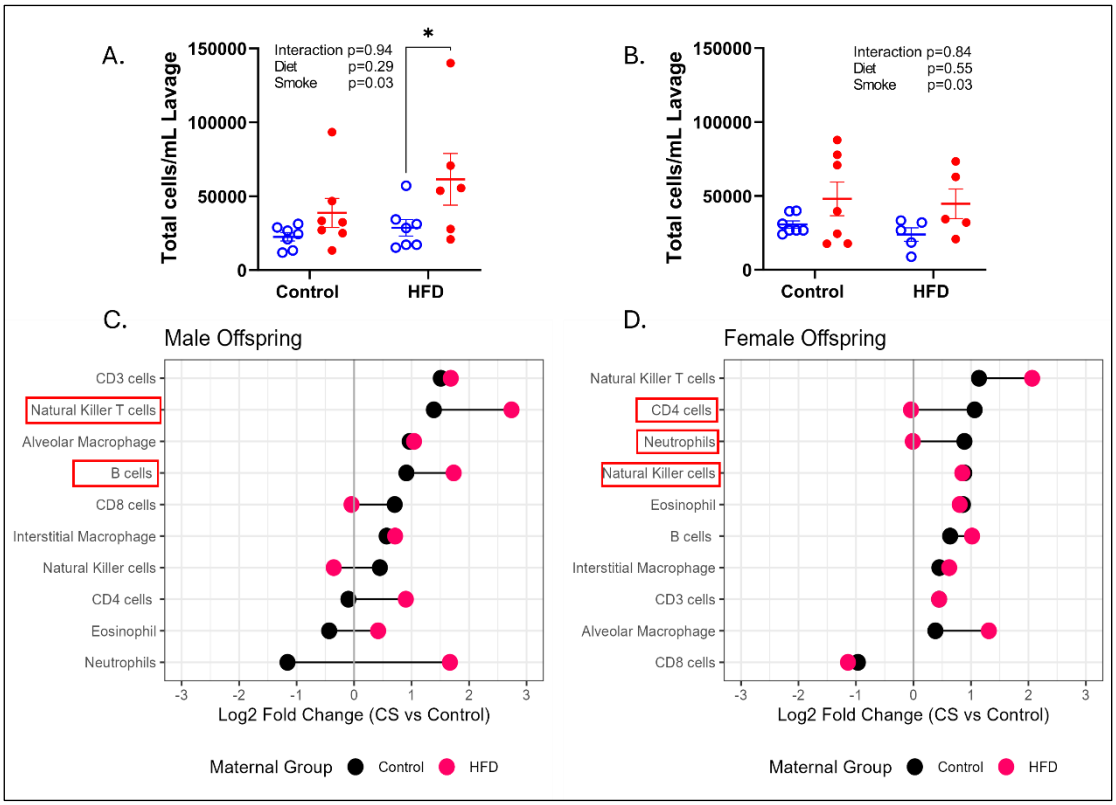


Figure 7: Maternal diabetes alter immune cell infiltration in lung in response to CS. Total cells in males (A) and females (B). Dumbbell charts illustrate CS-induced fold changes in immune cells for (C) male and (D) female offspring from control and HFD mothers. The y-axis represents immune cells, x-axis represents the log2 fold change of mean of immune cells in BALF. Black points represent the control group (Control-CS/Control), and pink points represent the HFD group (HFD-CS/HFD-Control). Overlapping points indicate no difference between groups and were excluded from statistical analysis (see **Figure 8** for statistical details). N=5-7 per group. The red highlighted boxes indicate significant differences found in cells, based on two-way ANOVA with Fisher's LSD multiple comparison test.

In all offspring, alveolar macrophages were the most abundant immune cell type, (over 40% of total cells), but with no significant exposure or sex-specific variations. To quantify the fold changes in differential cell counts in response to CS challenge, we calculated the ratio of each cell count for CS-exposed mice relative to the average cell count of their respective control counterparts. Male HFD offspring exposed to CS had significantly elevated Natural Killer T cells (+339%, p=0.03) and B cell counts (+332%, p=0.0004) compared to control HFD, and B cells were also significantly higher in HFD-CS than control-CS (+200%, p=0.007) (**Figure 8**A & B). The difference we see in neutrophil count in male HFD-CS offspring (**Figure 8**C) is largely being driven by three samples with high numbers. Also, both CD4 cells (+155%, p=0.14) and CD8 cells (+376%, p=0.23) were higher in HFD males compared to control diet, but with no difference after CS exposure (**Figure 8**D & H).

In female HFD offspring, there is a suppression of specific immune cells compared to control. After a 4-day CS challenge, HFD females showed significant reductions in Neutrophils (-32%,), CD4+ T cells (-21%), and Natural Killer (NK) cells (-47%) (p=0.03) compared to control CS (**Figure 8**K, L & M). The difference we see in NKT cell count in female control-CS offspring (**Figure 8**I) is largely being driven by two samples with high numbers. The increase of CD4+ T cells in HFD males and, reduction of CD4+ T cells in HFD females seen relative to control diet, independent of smoke exposure. This might hint a potential diet-related effects on CD4+ T cell accumulation. Alveolar macrophage accumulation increased with smoke exposure and females seemed to have a trend towards smoke effect (p=0.07). No real differences were observed in Eosinophil and CD8 cell counts in male offspring. But females had higher Eosinophil and CD8 counts than males, and HFD females showed reduced levels of these cell types in response to CS. Overall, males exhibited augmented immune responses, while females had suppressed immune responses. These findings suggest that the maternal diabetes may modulate responses to cigarettes in early adulthood in a sex-specific manner.

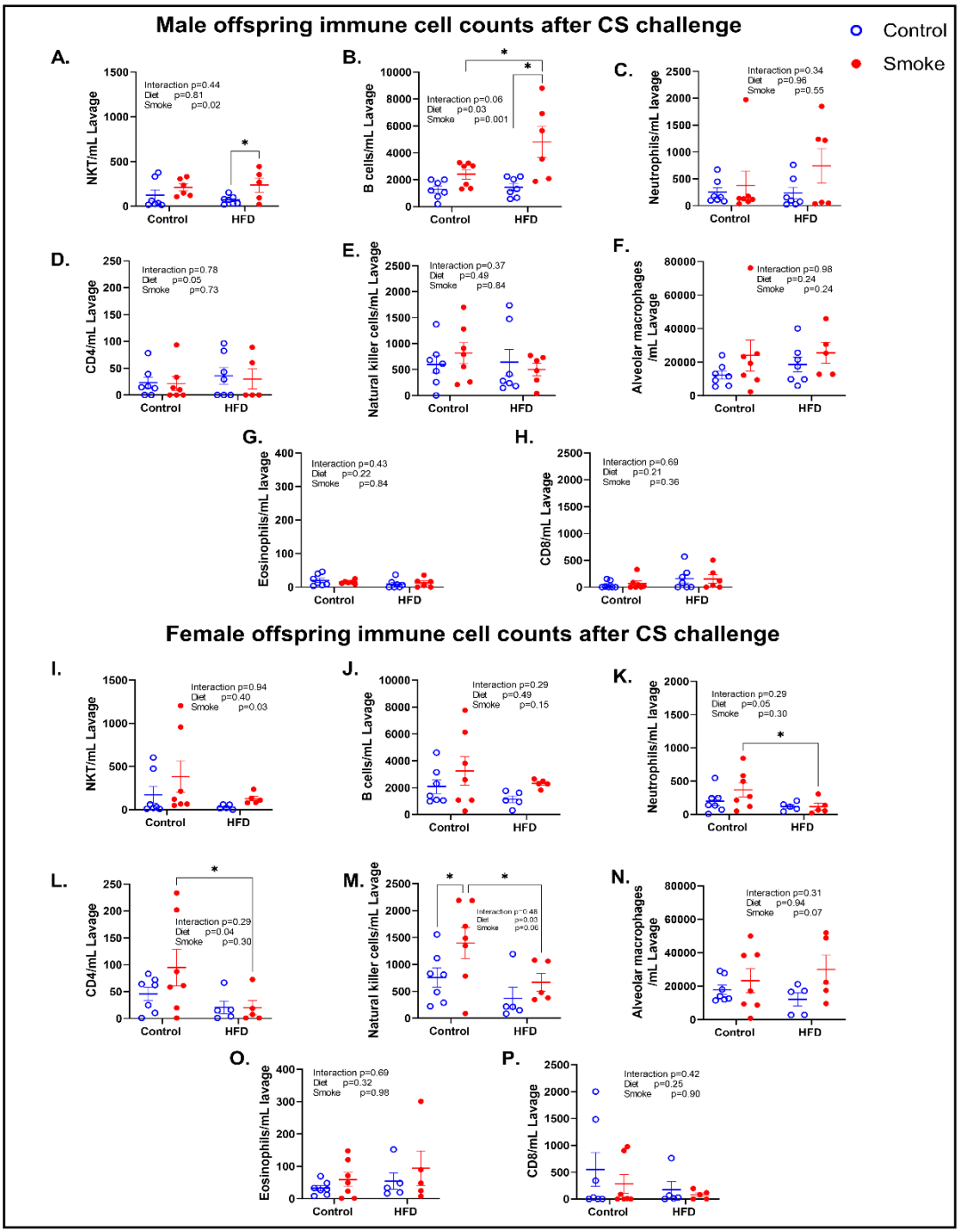


Figure 8: Exposure to maternal diabetes alter immune cell infiltration in offspring following 4-day CS challenge. HFD males have an augmented immune response to CS and HFD females have a suppressed immune response to CS. Upper panels (A-H) represents male offspring and lower panels (I-P) represents female offspring. (A,I) Natural killer T cells/mL, (B,J) B cells/mL, (C,K) neutrophils/mL, (D,L) CD4 cells/mL, (E,M) Natural killer cells/mL, (F,N) Alveolar macrophages/mL, (G,O) Eosinophils/mL, (H,P) CD8 cells/mL lavage. BALF samples were collected 24 hrs after the last exposure and data were normalized to the lavage volume. Here each dot represents an individual mouse. Analysis was done in a sex disaggregated manner, with smoke and diet as the factors. Statistical significance was determined using Two-way ANOVA with Fisher's LSD multiple comparison test (*P≤0.05). N=5-7 per group.

3.5 Sex related differences in Cytokine profile

To define sex-specific differences in the lung cytokine profile, we quantified the abundance of cytokines, chemokines and MMPs in mouse BALF samples. From downstream analysis, 27 mediators were included in the analysis. We observed sex- and exposure-specific variations in the cytokine profile at baseline and following a 4-day CS challenge.

Statistical analysis revealed that diet exposure significantly affected MMP-12 abundance (-51%, p value for diet p=0.002) in males and MIG/CXCL9 abundance (+268%, p value for diet p=0.007) in females, independent of smoke exposure. CS exposure significantly influenced Eotaxin and IL-1α abundance in both males and females (**Figure 10**). In female mice, Eotaxin abundance had a trend towards smoke effect (p=0.09). A significant interaction effect on IL-1α abundance in BALF was observed in both males and females (p=0.04). Following CS challenge, Eotaxin abundance significantly decreased (-55%, p=0.01) in control male offspring (**Figure 10**A), while IL-1α (+211%, p=0.004) and TGF-β1(+168%, p=0.03) were significantly increased (**Figure 10**B & H). In control female offspring, CS challenge led to a significant increase in Eotaxin (+199%, p=0.004) (**Figure 10**I) and a significant decrease in IL-1α (-45%, p=0.005) (**Figure 10**J). These results indicate sex-specific differences in Eotaxin and IL-1α profiles in control offspring in response to CS. These CS induced changes seen in the control animals, were blunted across both male and female HFD exposed offspring. No significant differences were observed in the abundance of MCP-1, MIP-1α, and MMP-3 in BALF for either sex at baseline or following CS exposure (**Figure 10**).

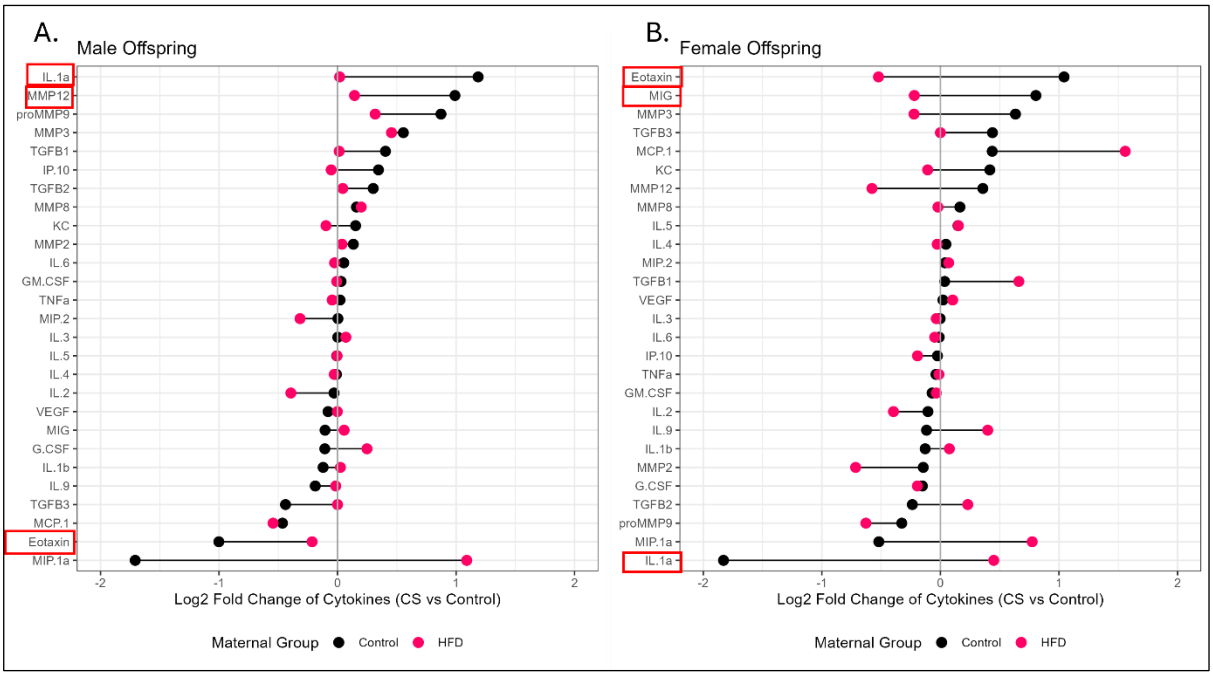


Figure 9: Maternal diabetes modifies the responses to CS and alter cytokine abundance in offspring lung. Dumbbell charts illustrating CS-induced fold changes in cytokine abundance for (A) male and (B) female offspring from control and HFD mothers. The y-axis represents individual cytokines analysed, while the x-axis shows the log2 fold change of median cytokine abundance in BALF of C57BL/6NJ mice. Black points represent the control group (Control-CS/Control), and pink points represent the HFD group (HFD-CS/HFD-Control). Overlapping points indicate no difference between groups and were excluded from statistical analysis (see **Figure 10** for statistical details). N=5-7 per group. The red highlighted boxes indicate significant differences found in cytokines, based on two-way ANOVA with Fisher's LSD multiple comparison test.

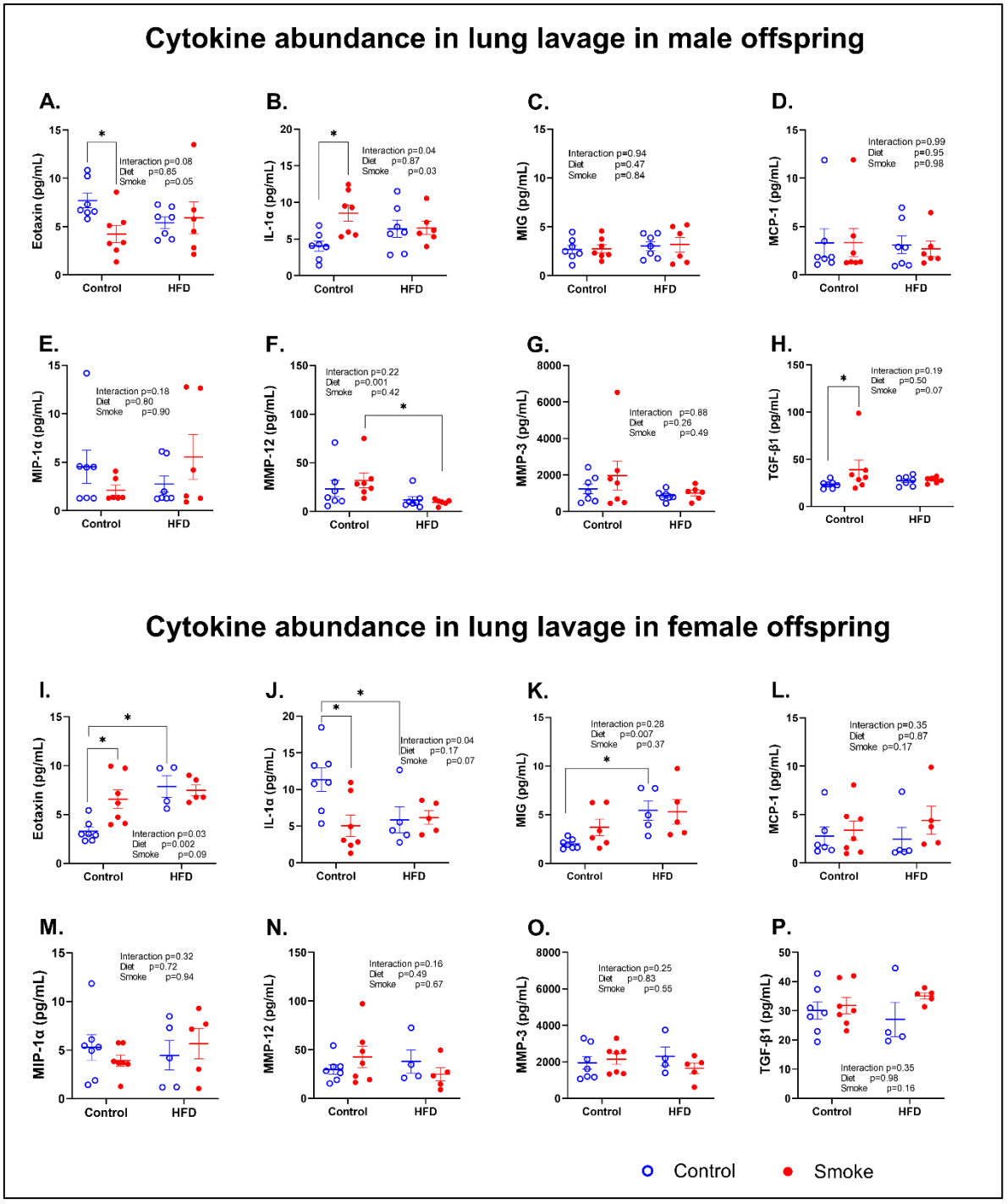


Figure 10: Maternal diabetes induces sex-specific alterations in offspring lung cytokine abundance following 4-day CS exposure. Eight-week-old offspring from diabetic pregnancies exhibited sex-specific differences in their cytokine profiles. Panels show cytokine abundance in BALF (pg/mL) of: (A, I) Eotaxin, (B, J) IL-1α, (C, K) MIG/CXCL9, (D, L) MCP-1, (E, M) MIP-1α, (F, N) MMP-12, (G, O) MMP-3, and (H, P) TGF-β1. Upper panels (A-H) represent male offspring, while lower panels (I-P) represent female offspring. Data were not normalized to lavage volume due to lack of correlation between cytokine abundance and lavage volume. Each dot represents an individual mouse. Analysis was conducted in a sex-disaggregated manner, with statistical significance determined using Two-way ANOVA with Fisher's LSD multiple comparison test (*P≤ 0.05). N=5-7 per group. HFD, high-fat diet.

3.6 DNA Methylation and Gene Expression

In this study we investigated DNA methylation (DNAm) differences at CpG sites in the promoter regions of 11 genes in the lung tissue of 8 to 10-week-old offspring to study the effect of maternal diabetes on offspring lung health (Figure 8). No significant differences were observed in DNAm of offspring from diabetic pregnancy compared to the control. However, we found a significant sex-specific effect on *Ahrr* DNAm at CpG [Chr13:742927342] (p=0.03) regardless of the diet. *Ahrr* DNAm was significantly higher in control males (88.95 ± 1.04%) than in control females (86.63 ± 1.04%), p=0.04. In both male and female offspring from diabetic pregnancy, we observed numerically reduced methylation in *Cyp1a1*, *Ephx1*, *Gstp1*, *Igfbp3*, and *Gstm1* in the lung compared to their respective controls that did not reach significance. Offspring exposed to maternal diabetes had numerically, but not significantly, increased *Igfbp1* DNAm, regardless of sex. Also, we combined both male and female samples together to increase the power, where there were no apparent sex specific differences in the responses. There were no significant differences were seen in DNAm in offspring from HFD dams compared to control (see **supplementary figure 2**).

Next, we measured gene expression of the Igf axis and PAH metabolism genes. Statistical analysis revealed that both diet (p=0.02) and cigarette smoke (CS) exposure (p=0.01) significantly affected *Igfbp3* gene abundance in males. In females, there was a trend towards a smoke effect (p=0.08). Male HFD offspring (-0.53 log2 fold change, p=0.03) had a significant reduction in *Igfbp3* abundance compared to control males. Following CS exposure, *Igfbp3* gene abundance further decreased in both sexes. CS exposure significantly reduced *Igfbp3* in control males (-0.58 log2 fold change, p=0.03) but did not reach statistical significance in females (p=0.33). CS exposure significantly influenced *Cyp1a1* abundance in both males and females (p=0.0001 and p=0.009 respectively), regardless of diet exposure. In male control (+2.76 log2 fold change, p<0.0001) and HFD (+1.62 log2 fold change, p=0.008) offspring showed a significant increase in *Cyp1a1* levels in response to CS, compared to their relative controls. This fold induction is less in HFD males than the controls (2-fold difference in induction). In male offspring, *Ahr* and *Gstt1* showed a trend towards an interaction effect (p=0.06 and p=0.09 respectively) with HFD-CS showing a decrease *Ahr* abundance and an increase *Gstt1* abundance relative to HFD control. In females, Ephx1 demonstrated a significant interaction effect (p=0.03) and diet effect (p=0.02). Additionally, HFD females exhibited a significant induction of *Ephx1* abundance following CS exposure (0.79 log2 fold difference, p=0.01).

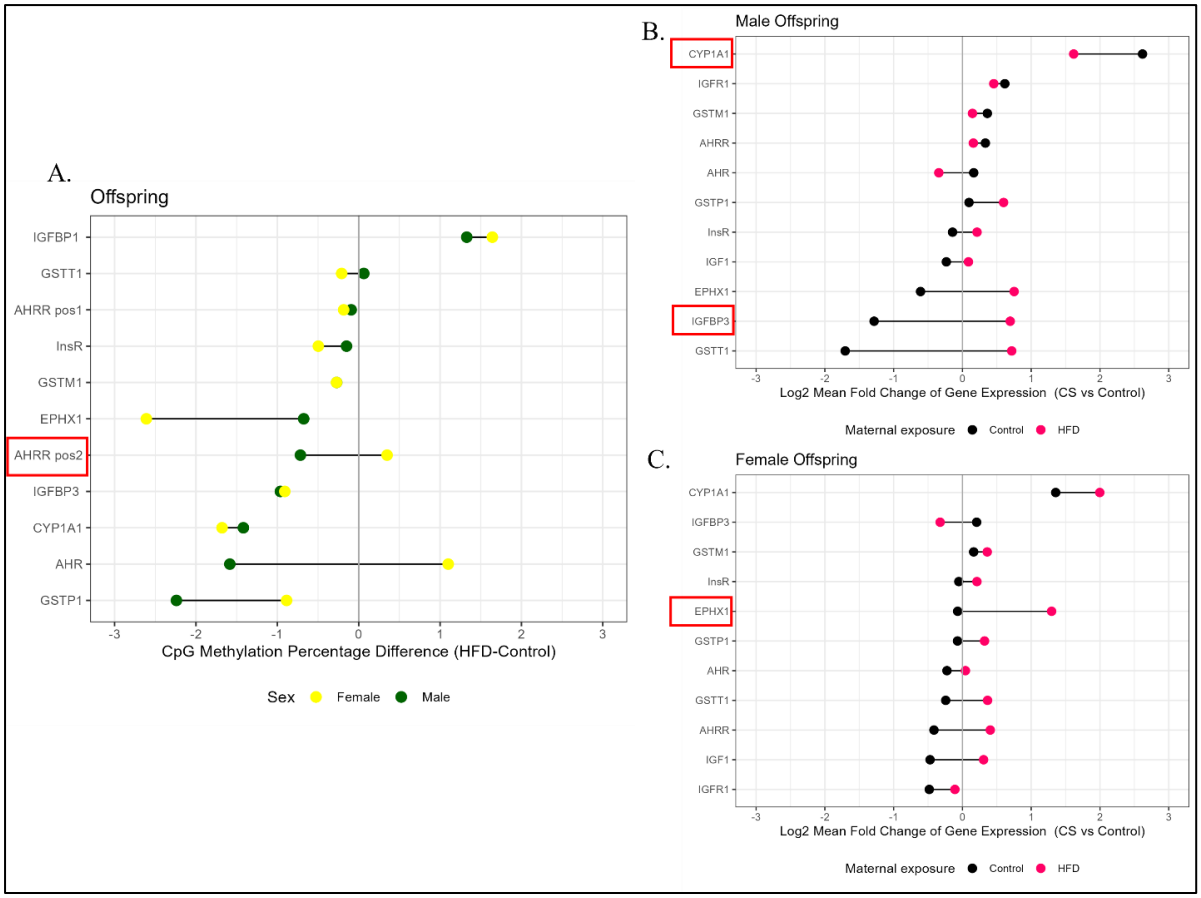


Figure 11: Maternal diabetes alters DNAm and gene expression in offspring. (A) illustrates the HFD induced DNAm changes in Igf axis and PAH metabolizing enzymes. Y-axis represents individual genes, while the x-axis shows the CpG methylation percentage difference (percentage methylation in HFD-percentage methylation in control). Green points represent males, and yellow points represent the females (N=4 per each exposure group). CS induced fold changes in gene abundance in Igf axis and PAH metabolizing enzymes (B) male and (C) female offspring from control and HFD fed dams. Y-axis represents individual genes. X-axis shows the Log2 fold change of gene expression. Black points represent the control group (Control-CS/Control), and pink points represent the HFD group (HFD-CS/HFD-Control). Overlapping points indicate no difference between groups, and points lied on or around x=0 means there's no differences in fold change, so those were excluded from statistical analysis (see **Figure 12** for statistical details). N=5-7 per group. The red highlighted boxes indicate significant differences found, based on two-way ANOVA with Fisher's LSD multiple comparison test.

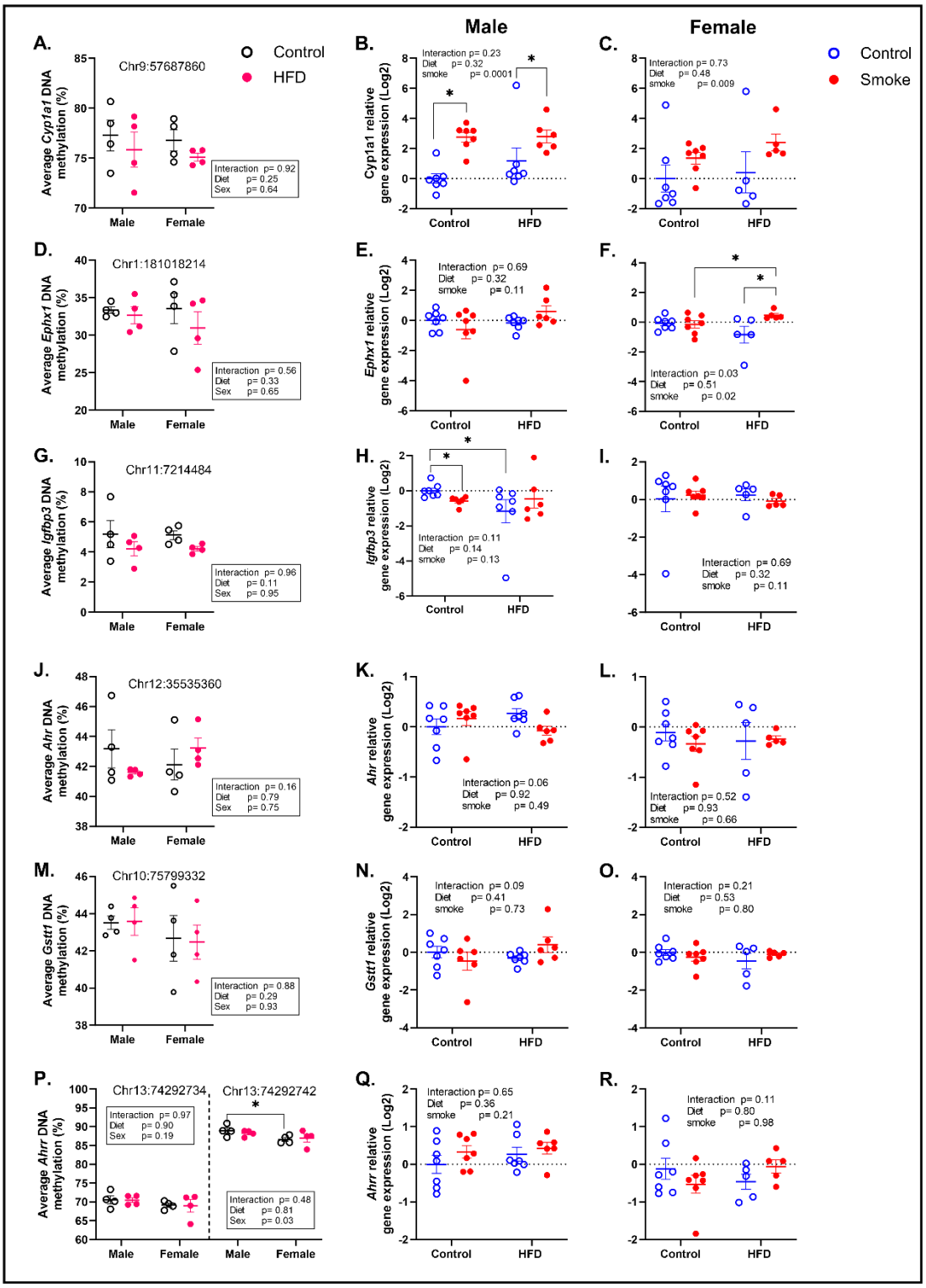


Figure 12: Maternal diabetes alters offspring DNA methylation and gene expression in Igf axis and PAH metabolizing enzymes following 4-day CS exposure. (A) *Cyp1a1* percentage DNA methylation in offspring lung at 8-10 weeks of age; *Cyp1a1* gene expression in offspring lung (B) male, (C) female. (D) *Ephx1* percentage DNA methylation in offspring lung; *Ephx1* gene expression in offspring lung (E) male, (F) female. (G) *Igfbp3* percentage DNA methylation in offspring lung; *Igfbp3* gene expression in offspring lung (H) male, (I) female. (J) *Ahr*

percentage DNA methylation in offspring lung; *Ahr* gene expression in offspring lung (K) male, (L) female. (M) *Gstt1* percentage DNA methylation in offspring lung; *Gstt1* gene expression in offspring lung (N) male, (O) female. (P) *Ahrr* percentage DNA methylation in offspring lung; *Ahrr* gene expression in offspring lung (Q) male, (R) female. Lung tissues were collected after 24 hrs of last CS exposure. Here each dot represents an individual mouse. statistical significance determined using Two-way ANOVA with Fisher's LSD multiple comparison test (*P≤ 0.05). DNAm was analysed using diet and sex as the factors. Gene expression analysis was conducted in a sex-disaggregated manner, with diet and smoke as the factors. N=5-7 per group. HFD, high-fat diet.

CHAPTER 4: DISCUSSION

COPD is a significant global health concern, with smoking identified as its primary cause. However, emerging research points toward the critical role of early-life exposures in shaping lung development and influencing COPD risk later in life. This paradigm shift underscores the importance of understanding the impact of environmental stressors during pregnancy and childhood, including exposure to tobacco smoke, air pollution, pre-term birth, bronchopulmonary dysplasia, and childhood asthma. Notably, maternal diabetes during pregnancy has been linked to impaired fetal lung development and other risk factors for COPD development, suggesting that it may be a potential risk factor for COPD that has yet to be explored. Studying the influence of environmental factors on lung health in adulthood is challenging in people due to the extended time between the prenatal period and the diagnosis of COPD (more than 50 years), and the potential for additional confounding exposures during this period. The use of animal models and controlled environmental exposures can provide important experimental support long-term human cohorts for this research. It is also important to recognize that genetic (heritability) factors play a crucial role in COPD pathogenesis, emphasizing the need for a comprehensive understanding of the interplay between genetics and environmental exposures.

From previous studies, we know that prenatal and early life factors can increase the sensitivity to cigarette smoke in adulthood, potentially making individuals more susceptible to future COPD (Drummond et al., 2017; Onuzulu et al., 2023; Upton et al., 2004). Up to date there is no research on whether exposure to maternal diabetes can also directly influence offspring responses to cigarette smoke, as a mechanism for future COPD. To our knowledge this is the first study to investigate the relationship between exposure to MD and risk for COPD later in life. Using a mouse model of prenatal diabetes, I explore changes in offspring sensitivity to CS induced lung damage.

In this study we observed that the exposure to maternal diabetes increase total lung resistance and Newtonian resistance in HFD male offspring following CS, suggesting that HFD males are more susceptible to CS induced lung dysfunctions. In terms of inflammation, we found HFD males seems to have an augmented inflammatory response (elevated B cells, NKT cells and IL-1α) and HFD females seems to have an immune suppression (reduction of neutrophils, $CD4^+$ T cells, NK cells and IL-1α) following acute CS exposure. Hinting at a possible mechanism, we found changes in gene expression relating to lung growth and repair, and

Polycyclic Aromatic Hydrocarbon (PAH) metabolism pathway were altered in HFD offspring in response to CS.

Glucose tolerance test is the most used test to quantify glucose intolerance and diabetes in a genetically modified or diet induced mouse model. Previous research shows that HFD fed female mice and rats (dams to be) have significantly increased maternal body weight, weight gain, and fasting blood glucose, and impaired glucose and insulin tolerance prior to breeding compared to control mice (Liang et al., 2010; Pascoe et al., 2022; Pereira et al., 2015). I see similar glucose intolerance and weight gain in the dams this study, confirming establishment of the model.

Current literature shows that, exposure to maternal HFD alters offspring lung function parameters including total lung resistance, lung compliance, inspiratory capacity, tissue damping and elastance (Griffiths et al., 2016; Heyob et al., 2019; Pascoe et al., 2022). The basal differences we have seen in this study are different from previously published results by our group. That may be related to differences in offspring body weight in this study and previous study, as we know there is a correlation between these lung function parameters and offspring body weight. In this study we found male specific differences in lung function in response to CS exposure. Male offspring from diabetic pregnancy had significantly increased total lung resistance and Newtonian resistance following CS. Total lung resistance represents a combination of lung resistance (airway resistance plus lung tissue resistance) and the resistance of the chest wall (Czovek, 2019). Newtonian resistance represents the resistance of the conducting airways (Cahill et al., 2022). Thus, the increase of this parameter suggests an airway narrowing. This could be due to mucus plugging (B. K. Huang et al., 2024) or active airway narrowing in response to CS (Higenbottam et al., 1980). So, males from HFD background have more airway narrowing in response to CS than do controls, which may cause breathing difficulties. We also find that males have altered lung compliance if they were exposed to maternal diabetes *in utero*. Measured with a PV-loop, the deflation curve is elevated, indicating gas trapping, possibly due to changes in lung compliance and elastic recoil in the lung. This is classically emphysema, which is characterized by enlargement of air spaces, increased lung compliance and decreased elastic recoil. Based on our lung function data, males seem to be more susceptible to emphysematous and bronchitic changes in COPD when exposed to maternal diabetes.

COPD is also characterized by lung inflammation that can contribute to progressive and irreversible airflow obstruction. In this study I demonstrated HFD influenced CS induced inflammatory responses in a sex specific manner. Males have heightened inflammatory response from total cells, B cells, and NKT cells, as well as some specific cytokines and chemokines. Females appear to have broad immune suppression in neutrophils, NK cells, CD4 cells, and IL-1α. Inflammation is believed to be the central for COPD, and this mainly characterized by infiltration of immune cells including neutrophils, macrophages and lymphocytes (Rennard, 2007; Y. Wang et al., 2018). These infiltrating immune cells release inflammatory mediators such as cytokines and chemokines (IL-1, IL-4, IL-5, IL-8, TNF-α, MMPs, TGF-β, MCP-1), as well as destructive enzymes, where these lead to lung destruction in COPD (Y. Wang et al., 2018).

Neutrophils are the most prevalent inflammatory cells found in the lumen and bronchial wall of a COPD patient (Hoenderdos & Condliffe, 2013). Rate of lung function decline correlates with airway neutrophilia (Stănescu et al., 1996). In smokers elevated neutrophil counts in the sputum is correlated with high resolution computed tomography measurements of peripheral airway dysfunction (O'Donnell et al., 2004). Furthermore, elevated neutrophilic inflammation is a hallmark of acute COPD exacerbations (Gompertz et al., 2001). Additionally, neutrophil count in the lung is dramatically increased in smokers compared to non-smokers (O'Donnell et al., 2004). In this study I have seen elevated neutrophils only in female offspring from control diet after CS. I saw no increase in neutrophils in HFD female in response CS, instead, there was a significant reduction of neutrophils in HFD-CS females compared to the control-CS females. The reduced neutrophils in females have not been explored in human COPD. It is unclear whether this suppression is beneficial or detrimental to COPD development. Neutrophil depletion during CS exposure led to a drastically increased pro inflammatory cytokines release in lung. The elevated MMP-12 suggest that neutrophil depletion leads to an enhanced macrophage activity, leading to an exacerbated inflammatory response (Milad et al., 2021). The possibilities for the impairment that we have seen in this study could be an impairment of neutrophil migration or early/delayed peak response. Neutrophil migration to the lung following allergen peaks at 8-hours, and may follow similar kinetics with cigarette smoke, in which case we have missed the peak in our data (Piyadasa et al., 2016). It is also possible that there is increased apoptosis of neutrophils in female offspring from diabetic pregnancy. Further studies need to understand the underlying mechanism for neutrophilic suppression in female

HFD-CS offspring. Also, IL-1α promotes neutrophilic infiltration into the site of infection and it is unclear whether the neutrophilic suppression I have seen here is related to the supressed IL-1α noted in my results.

B cells play a vital role in COPD pathophysiology. They have both beneficial and deleterious activities in COPD. B cells help to eliminate pathogens by activating the adaptive immune responses during acute exacerbations (Polverino et al., 2016). Beyond protecting against pathogens, B cells also known to produce autoantibodies. Where these autoantibodies act against lung tissues and extracellular matrix proteins leading to lung inflammation and tissue damage in COPD patients (Feghali-Bostwick et al., 2008; S. H. Lee et al., 2007; Núñez et al., 2011; Polverino et al., 2016). Additionally in a COPD lung, B cells promote the production of pro inflammatory cytokines such as IL-6 to further contributing to the disease progression (Polverino et al., 2016). Both animal and human studies have shown B cells are more strongly linked to the emphysema phenotype (Polverino et al., 2016). Increased number of B cells were reported in COPD small airways (Hogg et al., 2004) and large airways (Gosman et al., 2006) and correlated with COPD severity. In this study I found an elevated number of B cells in lung lavage of male HFD offspring following acute CS exposure. This increased B cells may promote chronic lung inflammation and destruction of lung tissues, suggesting male offspring from diabetic pregnancies are more susceptible to CS induced lung damages. Increased number of B cells correlate with number and size of lymphoid follicles in severe COPD (Polverino et al., 2015). So, in future we could assess B cell-derived autoantibodies and number/size of lymphoid follicles in lung to provide additional evidence for B cell involvement in development of COPD.

In this study I showed, at baseline (no CS) female offspring from diabetic pregnancy had increased the abundance of pro inflammatory mediators such as Eotaxin and MIG but reduced IL-1α in the lung lavage. Eotaxin is a selective eosinophil chemoattractant, and it is elevated in COPD patients and, more classically, asthma (Eltboli & Brightling, 2013). Increased levels of eotaxin may suggest a potential for eosinophilic inflammation in HFD-females. In COPD, elevated eosinophils may be related to co-existing asthma and may be a therapeutic target for reducing COPD exacerbations in a subset of high eosinophil-COPD (Eltboli & Brightling, 2013; Jose, 2018). In our model, CS in control animals increased eotaxin in females and reduced in males. Interestingly, these sex specific changes were blunted in HFD exposed offspring, with

both sexes showing no changes in eosinophils following CS, but females still showing elevation overall. Therefore, females exposed to HFD may have elevated eosinophil related inflammation that changes the inflammatory response to CS.

CXCL9/MIG (Monokine induced by interferon-γ) is a chemokine, mainly produce by alveolar macrophages (Donnelly & Barnes, 2006) and bronchial epithelial cells (Sauty et al., 1999). CXCL9/MIG act on CXCR3 receptor to attract T-lymphocytes and B-lymphocytes into the lung (Henrot et al., 2019). A human study found that CXCL9 concentration was increased in the sputum of COPD patient compared to nonsmokers and correlated with neutrophils (Costa et al., 2008). Another study reported both CXCL9 and its receptor CXCR3 are highly expressed in T cells and B cells in the lung lymphoid follicles of COPD patients. CXCR3 expression progressively increases with worsening COPD and inversely correlated with FEV1 (Kelsen et al., 2009). In this study I found significant elevation of MIG only in female offspring from diabetic pregnancy, independent of smoke exposure. This might suggest a potential for B cell and T cell ($CD4^+$ T cell, $CD8^+$ T cell) inflammation in the lungs of females exposed to HFD in utero. But I did not see any changes in B cell and T cell population in HFD females at baseline (no CS). This may be due to impairment of B cell and T cell migration from lung tissues to the lavage or may be due to early/delayed activation. CXCL9 is a secreted marker of M1 macrophages, which tend to be proinflammatory (Través et al., 2012). But it is unclear whether maternal diabetes alter the inflammatory state of macrophages in the lung.

IL-1α is a proinflammatory cytokine that plays an important role in the initiation and persistence of inflammation in COPD (Botelho et al., 2011). Most of the previous studies has demonstrated the role of IL-1β in COPD disease progression. But involvement of IL-1α and its role in COPD development remains less explored. In 2011, Botelho and others reported elevated levels of IL-1α and IL-1β in COPD patients and found a strong correlation between IL-1α and IL-1β in sputum samples from COPD patients with stable disease and at different acute exacerbation episodes (Botelho et al., 2011). This finding highlights the importance of IL-1α in COPD. Regardless of the sex, offspring from maternal GDM showed a lowest circulating IL-1α compared to its control counterparts (Mihalovičová et al., 2023). Similarly, I found a significant reduction of IL-1α in lung lavage specifically in HFD females, but not males, which may suggest a sex specific variation. Acute 4-day CS exposure significantly increases IL-1α in lung homogenates, and CS induced neutrophilia is dependent on IL-1α in

both acute and chronic CS exposure models (Botelho et al., 2011; Nikota et al., 2014). My results corroborate these findings in males, demonstrating that acute CS exposure significantly increases IL-1α level in active smokers of the control diet. However, IL-1α was significantly reduced in females following CS, a novel sex-specific finding. Interestingly no differences were seen in diabetic groups in response to CS, suggesting a blunting of the respective responses in control male and female offspring. It is unclear the mechanisms underlying these sex specific differences. Further research needs to investigate whether these changes are beneficial or detrimental in COPD pathogenesis. Furthermore, the reduced IL-1α in females has not been explored in human COPD. That is, whether this same reduction is seen in female smokers or COPD.

In this study I also demonstrated a suppression of CD4+ T helper cells in the lung lavage of female HFD-CS offspring compared to control-CS group. So, this suppression could be really a broad suppression of immune response in HFD-female or could be a delayed activation of CD4+ T cells in which case I have missed the peak. CD4+ T cells activate and, further differentiate into various T cell subsets including regulatory T (Treg) cells, T-helper (Th) cell types of Th-1, Th-2, Th-17 and Th-22. Among these Th-1, Th-2, Th-17 are closely linked with COPD (Brozyna et al., 2009; Bruzzaniti et al., 2019; Di Stefano et al., 2009). These cells specifically produce pro inflammatory cytokines and promote airway inflammation (Qin et al., 2022). CXCR3 receptor chemokines such as CXCL9, CXCL10 are important in recruiting CD4 T cells to the site of inflammation (Barnes, 2009). So, in future studies we could assess the level of these chemokines, to explain their involvement in the reduction of CD4 T cells in our experimental model. Furthermore, this CD4+ T cell reduction could be due to CD4/CD8 skewing. The ratio of CD4:CD8 is a good indicator of immune responses and T lymphocyte population (Bai et al., 2019). An altered CD4:CD8 ratio indicates impaired immune responses and shifts in T cell populations. Measuring the factors influencing CD4/CD8 skewing would be beneficial to have a better understanding of the mechanisms behind the reduction of CD4+ T cells in HFD offspring.

I also demonstrate an impairment of NK and NKT cell infiltration in individuals from diabetic pregnancy following 4-day CS challenge. Male offspring had higher NKT cell number whereas female offspring has supressed NK cell compared to the typical responses seen in control groups. NK and NKT cells produce pro inflammatory cytokines and play an important role in maintaining immune system homeostasis (Hervier et al., 2019; Hodge et al., 2013). COPD

patients have higher numbers of NK and NKT cells, which suggests that these cells play a major role in the elevated levels of pro-inflammatory cytokines that have been linked to COPD (Hodge et al., 2007). TNF-α is a pro inflammatory cytokine known to be involved in the development of COPD (Barnes et al., 2003). It has been demonstrated that it's induction in the lung can cause emphysema in a mouse model (Churg et al., 2004). TNF-α induce the production of IFN-γ from T cells and activate Immune cell infiltration (Hodge et al., 2013). One study demonstrated that CS could stimulate NK and NKT cells to produce IL-17A, where IL-17A is a proinflammatory cytokine that regulate neutrophilic inflammation in the airways and lung remodelling (Ritzmann & Beisswenger, 2021). Another study found a negative correlation between IL-17A concentration in serum and FEV1% predicted in COPD patients (Zou et al., 2017). Taken together, these cytotoxic NK and NKT cells may contribute to lung function impairment, lung injury, thereby playing a role in the pathogenesis of COPD.

Previous studies have shown that *in-utero* exposure to CS alter DNAm changes in newborn umbilical cord blood, placenta, nasal epithelia, lung tissues and adult pheripheral blood granulocytes (Breton et al., 2009; Joubert et al., 2016; K. W. K. Lee et al., 2015; Onuzulu et al., 2023b; Reddy et al., 2021; Suter et al., 2011). Similarly, some other studies also have shown maternal diabetes associated with DNAm in placenta, cord blood and pancreatic cells (Finer et al., 2014; Howe et al., 2020; Słupecka-Ziemilska et al., 2020). However, up to date there are no studies to measure the effect of maternal diabetes exposure and early adult smoke exposure in the lung. In this study I measured changes in DNA methylation in the promoter regions of Igf1, insulin receptor (Ir), Igfbp1, Igfbp3 (Igf1 signaling axis) and Cyp1a1, Ephx1, Gstm1, Gstt1, Gstp1, Ahr, and Ahr repressor (PAH metabolism axis). Igf1 members and PAH metabolism enzymes are normally induced by CS exposure to promote tissue repair and detoxify PAH respectively. In this study I found maternal diabetes did not significantly alter DNAm in offspring lung at our selected CpGs in promoter regions of the genes involved in Igf axis and PAH metabolism pathway. However, I did find significant changes in the abundance of a key gene related to lung growth.

Cytochrome p450 A1 (CYP1A1) is one of the main enzymes in human lungs and known to be primarily responsible for metabolism and activation of procarcinogens and xenobiotics found in the cigarette smoke and environmental exposures. HFD offspring showed a slight, but not significant decrease in Cyp1a1 DNAm, which might suggest a potential for higher gene

expression in offspring lung. Previous studies have found that the expression level of CYP1A1 was significantly higher in smokers compared to its control non-smokers (J. H. Kim et al., 2004; Mollerup et al., 2006). Our results align with these findings, and I observed a higher level of Cyp1a1 abundance in both control and HFD mouse lung challenged with CS. Changes in Cyp1a1 expression showed no relation to maternal diabetic exposure. Detoxification is the physiological function of Cyp1a1. Cyp1a1 produce ROS during its catalytic cycle (Haag et al., 2002; Puntarulo and Cederbaum 1998) and is linked with oxidative stress (Park et al., 1996; Shertzer et al., 1998). Oxidative stress promotes chronic lung inflammation and stimulate mucus secretion which contributes to airway obstruction in COPD patients (Barnes, 2022).

The results of this study highlight a suppression of Igfbp3 mRNA abundance in HFD-male offspring at baseline and after challenged with acute CS exposure. Igfbp3 is involved in Igf1 axis, and evidence suggests that Igfbp3 play a crucial role in lung development and cellular repair processes in response to lung injury (Z. Wang et al., 2018). There is a positive correlation between Igfbp3 level and FEV1 and FVC in adulthood (Gläser et al., 2009). Low levels of Igfbp3 are linked with lung function reductions (Gläser et al., 2009; Green et al., 2014) where impaired lung function is a hallmark feature of COPD pathophysiology. Further, Igfbp3 play a role in the production of extracellular matrix components in the lung (Pilewski et al., 2005). Reduced Igfbp3 could change the composition of the lung, and in males where this difference is seen, may account for the structure-function differences we see in the PV Loops.

In this study I found a significant elevation of microsomal epoxide hydrolase (Ephx1) abundance in HFD-female smoking individuals. Here the change is small in females, but from HFD control is still a 1.79-fold induction after CS and no change in control. Ephx1 is an enzyme involved in the metabolism of toxic compounds in CS and found to have a role in COPD pathogenesis. Ephx1 primarily expressed in lung epithelium, which metabolize epoxides in CS, potentially modulating airway inflammation and oxidative stress. Genetic polymorphism in EPHX, in exon 3 (substitution of tyrosine to histidine) and exon 4 (substitution of histidine to arginine) correlates with enzyme activity ((Vibhuti et al., 2007)). Slowest activity haplotype (113H-139H) was associated with imbalanced oxidative stress and decreased FEV1 (Vibhuti et al., 2007). A study in U.K population found that the slowest activity haplotype was linked with COPD and emphysema (Smith & Harrison, 1997), and this was associated with more severe COPD in a Japanese population (Yoshikawa & Yamakido,

2000). This dysregulated gene expression may modulate individual's susceptibility towards the development of COPD.

Importantly, I did not see significant changes in DNAm in our animal lung tissues and there was no correlation found between DNAm and gene expressions. Therefore, the changes I have seen in *Cyp1a1*, *Ephx1* and *gfbp3* gene expression could be due to the involvement of other types of epigenetic modifications, or methylation at other sites within the promoter region. So, in future we could use whole genome bisulfite sequencing to obtain a comprehensive view of DNAm across the entire genome. The offspring used in this study are still too young to be diagnosed for COPD, and COPD in mice is difficult to model. However, by measuring changes in lung function, inflammation, DNAm and gene expression in HFD mice following acute CS, we can potentially identify changes in sensitivity and determine whether these changes align with changes we observe in COPD.

4.1 Overall Conclusion

In this project the main goal was to understand whether maternal diabetes alone or with combination of cigarette smoke represents a novel risk factor for COPD. Here I highlight that even before CS exposure, male offspring from diabetic pregnancy had the most compliant lungs with a higher pressure-volume loop area. Which may suggest an altered lung phenotype in male mice from HFD dams. Further my data highlight male offspring from diabetic pregnancies have altered lung function measurements following CS challenge. Specifically, there was an increased total lung resistance and small airway resistance following CS, compared to the control group. Also, I found HFD influenced CS induced immune cell infiltration in a sex-specific manner. In female HFD-CS there was an immune suppression, a significant reduction of Neutrophils, CD4+ T cells and NK cells compared to control-CS. In HFD males I found an excessive B cell and NKT cell response following CS challenge where this could lead to a chronic lung inflammation in male mice. Moreover, at baseline I found HFD female offspring had elevated Eotaxin and MIG but reduced IL-1α. CS induced cytokine changes in control animals, which were sex specific, were blunted in HFD exposed offspring. Additionally, I observed changes in gene abundance, but without specific changes in DNA methylation in smoking individuals from diabetic pregnancy. Taken together all these findings presented here suggests that it worsens lung health in smoking individuals from diabetic pregnancy, specifically males. MD may modulate response to cigarettes in early adulthood, suggesting a propensity to future COPD.

Understanding the early life risk factors that drive future COPD can provide opportunities for primary prevention, such as smoking cessation, avoidance of environmental pollutants, and proper nutrition during pregnancy and pre-pregnancy periods. Infants who are exposed to tobacco smoke during pregnancy are more likely to develop COPD later in their life (Beyer et al 2009). Therefore, reducing infants' exposure to tobacco smoke by promoting the mothers to quit smoking either before or during her pregnancy would be an effective intervention (McEvoy & Spindel, 2017). According to a recent randomized clinical trial and studies in non-human primates, vitamin C supplementation during pregnancy may prevent some of the effects of in-utero smoke exposure on offspring respiratory health (McEvoy et al., 2014, 2023; Proskocil et al., 2005). The trial involved pregnant smokers who were unable to quit smoking and were given 500mg/day of vitamin C. The newborns of mothers who received vitamin C had significantly improved newborn pulmonary function tests, including increased passive respiratory compliance and improved tidal breathing measurements, compared to newborns whose mothers received a placebo. These findings suggest that vitamin C supplementation may be a safe, inexpensive, and simple intervention for pregnant women who cannot quit smoking to improve their infants' lung health. This example here highlights how a safe intervention can mitigate the risk of developing chronic lung disease following developmental exposures. Similarly for maternal diabetic exposures, safe measures such as maintaining optimal blood glucose levels and taking supplements or medications could potentially reduce the risk of chronic lung diseases and sensitivity to lung damaging factors like cigarette smoke in offspring. Additionally, proper nutrition during pregnancy can help ensure that the fetus develops healthy lungs. Pregnant women should follow a special meal plan with smaller, more frequent meals and take vitamins and nutritional supplements if needed.

4.2 Significance of this study

In history, the sex-based differences have not been considered in COPD research. But the emerging evidence shows that there are significant sex-based differences in COPD pathophysiology. In this study I did sex disaggregated data analysis, and the results shows sex specific responses in HFD offspring following CS exposure. These results will provide the foundation for future research to further understand the mechanisms that causes sex differences in lung dysfunction, inflammation and genetics in COPD disease progression.

It is challenging to study the impact of genetic or environmental factors on future lung health in human research. Because the length of time from in utero to until diagnosis of COPD is more than 50 years. As well as it is difficult to control the exposures over that length of time. In this study I used C57BL/6NJ mouse model and controlled environmental exposures to generate evidence to support long term studies in humans. The findings from this study can be used to inform research questions in human cohort data.

Typically, COPD occur in older individuals. But here I have used mice at age 8-10 weeks, where this is equivalent to 17-years old in humans. This is the age that many individuals first try cigarettes. Previous research indicates that the aging does not enhance the negative impact of cigarettes in mice (Zhou et al., 2013). So, here I can use CS induced responses in young HFD offspring, as an indicator of susceptibility towards COPD. Additionally, in this project we bio banked lung, blood, heart and cecum from offspring and mothers to support future investigations to explore mechanisms driving the observed changes in response to CS challenge. This is the first study to suggest that maternal diabetes modifies CS response and may predispose offspring to future COPD, specifically in males.

4.3 Limitations of the study

For this study all samples were collected at a single time point (24 hrs after the last CS exposure). So, this work presents a single snapshot of all the responses. Future studies need to be done at different time points following CS, specifically to assess differences in kinetics of immune cells. With the long exposure and breeding timeline to get data and the limited amount of lung tissue, we chose to focus on molecular markers and not collect tissue for histology. Therefore, we do not know if changes in lung function in offspring are due to changes in airway morphology or cell composition. Sex-related differences in airway remodeling were not assessed in this study. However, future studies could explore how these prenatal exposures change the structure of the airway and lung.

We have chosen a small set of genes to focus on in our epigenetic research in objective 2, limiting our ability to identify other novel pathways that may be impacted. However, DNA from the lung tissue were banked to allow for future analysis of whole genome methylation. In addition to that in this current study I have only focused on a single CpG location in the promoter regions. But CpGs can be differentially methylated in different CpG locations (See **Figure 12** for Ahrr DNAm). With the current study design, we have seen slight changes in DNAm. But we do not know whether these changes are due to direct effects of diabetes, or if they are due to some other in utero factors. Also, the current study has not looked for DNAm in offspring following CS challenge at 8-weeks of age. Even we do not see any significant changes with MD exposure, there could be a possibility to see altered DNAm profile in offspring after CS exposure. So further studies need to be done. Another limitation of this study was the small sample size, with a minimum sample number of n=5 per sex per exposure. So, in future to increase the offspring numbers we can increase the number of breeding mice (dams to be) for the prenatal exposures.

4.4 Future directions

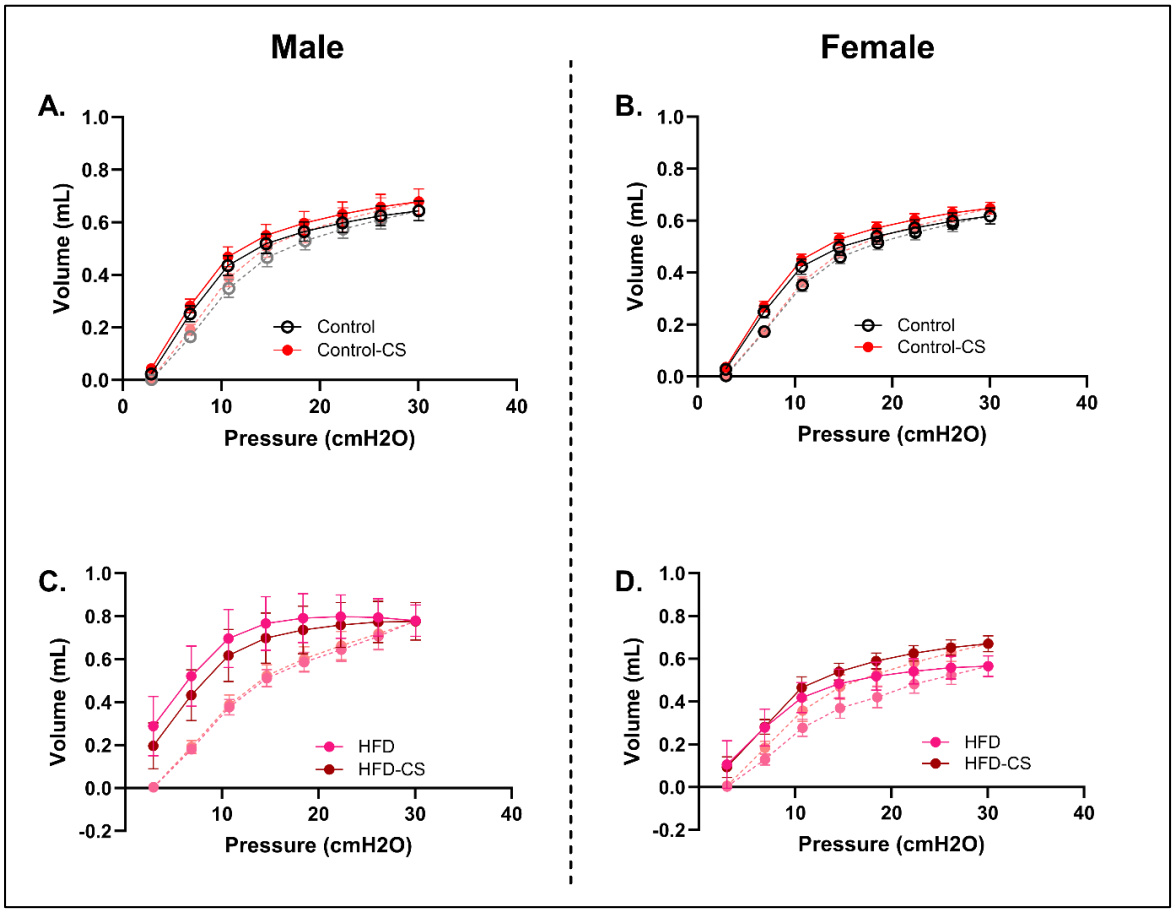
In this study I observed that prenatal exposure to MD has exacerbates CS induced lung dysfunctions. Additionally, MD has altered airway inflammation markers, including immune cells and cytokine profile in response to 4-day CS challenge. These changes indicate that offspring from diabetic pregnancies are more susceptible to CS induced lung dysfunctions and inflammation. Future research could explore the chronic CS exposure model to better replicate COPD features, including emphysema. In the present study I did not perform lung histology on offspring after CS exposure to assess the changes in airway morphology. However, the changes of lung function are typically correlated with histopathological changes in mouse models of COPD, serving as indicators of alveolar enlargement and airway remodelling. Importantly, this work will enable future studies to perform a detailed analysis of lung morphology using histology.

Interestingly, from this study I observed an unexpected suppression of neutrophils in BALF of female offspring from diabetic pregnancies following acute CS exposure. This observation was limited to a single time point (24 hrs after the last CS challenge). To better understand, it is important to investigate the neutrophil response at different time points following CS exposure (at 6, 12, 24, 48 and 72 hrs). This will be helpful to determine whether neutrophil infiltration peaks before or after 24 hr time point. Also, we can compare the neutrophil count in both lung and BALF to understand whether the observed suppression is due to impaired infiltration from lung tissues to the BALF. In the current study we don't see any significant changes in DNAm of genes in Igf axis and PAH metabolism pathways. So, this enables future studies to use whole-genome methylation analysis to identify previously unknown epigenetic mechanisms underlying the detrimental effects of MD.

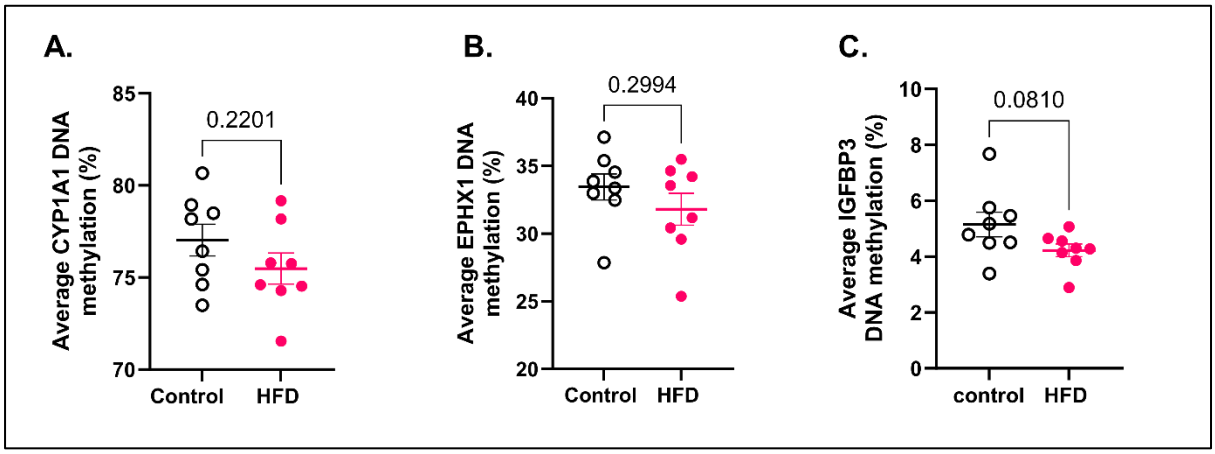
Co-exposure to MD and CS synergistically increases risk for asthma and pre-term birth (Azad et al., 2013; Borsari et al., 2018). According to epidemiologic data, 23% to 38% of mothers who have diabetes also smoke (Azad et al., 2013; Clair et al., 2013). It's important to note that communities with lower socioeconomic level, the smoking and diabetes rates are also higher. So, the rate of MD and CS co-exposure rates may be also higher in those populations (Bird et al., 2015). As a part of this current study, we collected data for offspring from prenatal CS exposure and prenatal MD and CS co-exposures to see their sensitivity towards CS induced lung damage. So, in future we can analyse these data to check the association between these exposures and future COPD risk.

CHAPTER 5: APPENDIX

Supplementary Figures



Supplementary Figure 1: PV loops showing the mechanics of the lung in response to 4-day CS exposure. Offspring from control dams (A) males, (B) females. Offspring from HFD dams (C) males, (D) females. N=5-7 per exposure. HFD-high fat diet, CS-cigarette smoke exposure.



Supplementary Figure 2: Prenatal exposure to MD alter DNA methylation of genes in Igf1 axis and PAH metabolism pathway. Percentage DNA methylation in offspring lung at 8-10 weeks of age (A) Cyp1a1, (B) Ephx1, (C) Igfbp3. Here I combined both male and female samples together to increase the power, where there were no apparent sex specific differences in the responses. N=8 per exposure.

Supplementary Tables

Supplementary Table 1: Total and differential cells in lung lavage of offspring from control and HFD diet in response to 4-day CS challenge. The red highlighted ones indicate significant differences found, based on two-way ANOVA.

	Control		Control-CS		HFD		HFD-CS	
Cell Differentials	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males								
Total cells	22491.5	7496.71	38733	26086.71	28662.14	14687.72	61429.58	42741.26
Neutrophils	249.21	224.57	377.35	704.46	233.44	283.81	741.29	792.07
Alveolar Macrophage	12222.77	6598.28	23897.47	24411.37	18513.38	11823.61	25420	13874.01
Eosinophils	20.44	17.1	15.14	5.65	9.5	13.38	12.69	13.87
Interstitial Macrophag	777.53	559.04	1150.82	609.8	585.73	230.69	960.42	621.36
NKT cells	123.26	159.17	208.73	96.79	70.12	47.63	236.58	176.7
NK cells	597.07	434.5	814.83	539.45	637.66	670.45	498.72	285.37
CD4	23	27.57	21.46	33.87	35.82	40.37	29.8	42.06
CD8	42.29	68.12	68.68	120.11	159.28	208.97	154.47	198.97
CD3	416.75	584.78	1185.41	1053.85	558.47	684.18	1791.22	2581.69
B cells	1279.12	684.79	2403.49	915.36	1446.88	723.26	4808.45	2784.91
Females								
Total cells	30670.64	6573.44	47991.79	30162.85	23881.49	10204.71	44661.91	22298.59
Neutrophils	199.35	176.2	368.63	284.07	118.94	62.07	117.99	114.38
Alveolar Macrophage	17831.93	7536.93	23233.43	18833.94	12070.67	8682.25	29964.07	19153.16
Eosinophils	32.39	20.99	59.11	57.61	53.84	56.28	94.06	119.53
Interstitial Macrophag	1645.06	1517.26	2247.58	1846.1	889.53	539.46	1368.66	690.94
NKT cells	173.45	253.72	382.27	485.29	30.67	26.96	128.28	64.05
NK cells	757.67	475.26	1398.4	759.01	370.62	463.13	667.83	369.18
CD4	45.36	32.63	94.74	89.1	20.54	26.62	20.14	30.2
CD8	551.48	836.98	282.27	450.08	174.35	330.25	79.18	82.88
CD3	1292.73	1659.86	1761.69	1757.99	562.49	917.86	766.59	1221.27
B cells	2082.93	1371.85	3241.26	2822.69	1138.38	580.91	2307.9	309.56

Supplementary Table 2: Cytokine abundance in lung lavage of male control and HFD offspring in response to 4-day CS challenge. The red highlighted ones indicate significant differences found, based on two-way ANOVA.

	Male Offspring							
	Control		Control-CS		HFD		HFD-CS	
Cytokine	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eotaxin	7.7	1.99	4.23	2.39	5.41	1.56	5.91	4.1
G-CSF	2.38	0.57	2.25	0.49	1.9	0.34	2.41	0.37
GM-CSF	1.49	0.59	1.19	0.06	1.51	0.67	1.27	0.2
IL-1α	4.05	1.85	8.55	2.97	6.4	3.15	6.52	2.26
IL-1β	1.46	0.34	1.16	0.1	1.27	0.21	1.23	0.12
IL-2	1.58	0.46	1.55	0.51	1.76	0.62	1.47	0.36
IL-3	1.11	0.05	1.14	0.05	1.12	0.11	1.14	0.02
IL-4	1.07	0.04	1.07	0.04	1.11	0.03	1.08	0.05
IL-5	1.25	0.08	1.29	0.12	1.29	0.18	1.23	0.15
IL-6	1.12	0.05	1.17	0.04	1.2	0.15	1.15	0.03
IL-9	11.55	3.51	11.43	2.54	13.41	2.75	12.81	3.97
IP-10	3.06	0.8	3.39	0.62	2.82	0.48	2.69	0.38
KC	3.25	1.7	3.43	1.61	3.62	1.16	4.18	1.71
MCP-1	3.3	3.87	3.34	3.91	3.1	2.44	2.7	1.92
MIG	2.7	1.11	2.77	1.09	3.04	1.19	3.18	1.87
MIP-1α	4.52	4.56	5.25	8.35	2.74	2.27	5.55	5.67
MIP-2	14.64	10.66	17.1	6.69	17.13	7.2	11.61	6.44
TNFα	1.2	0.12	1.16	0.04	1.19	0.12	1.14	0.05
VEGF	7.56	1.43	7.9	2.58	8.31	3.59	9.07	3.34
TGF-β1	23.22	4.11	38.82	27.18	27.1	5.14	27.83	3.25
TGF-β2	13.96	3.8	17.09	6.73	16.96	2.49	17.04	4.23
TGF-β3	0.5	0.11	0.28	0.21	0.41	0.22	0.48	0.36
MMP-2	1580.18	877	3363.62	4537.76	1104.04	469.19	1056.58	425.8
MMP-3	1236.73	734.07	1964.26	2126.3	861.87	268.81	1011.53	373.5
MMP-8	1952.2	215.73	2923.13	1441.75	2158	604.37	2324.37	369.93
MMP-12	23.38	22.45	31.89	20.08	11.99	9.1	9.54	2.98
proMMP-9	83.21	36.45	171.69	158.1	99.74	40.65	110.94	40.73
IFNγ	OOR							
IL-7	OOR							
IL-10	OOR							
IL-12p40	OOR							
IL-12p70	OOR							
IL-13	OOR							
IL-15	OOR							
IL-17	OOR							
LIF	OOR							
LIX	OOR							
M-CSF	OOR							
RANTES	OOR							
MIP-1β	OOR							

OOR-Out of Range

Supplementary Table 3: Cytokine abundance in lung lavage of female control and HFD offspring in response to 4-day CS challenge. The red highlighted ones indicate significant differences found, based on two-way ANOVA.

	Female Offspring							
	Control		Control-CS		HFD		HFD-CS	
Cytokine	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eotaxin	3.3	1.1	6.55	2.53	12.03	9.46	7.48	1.22
G-CSF	2.43	0.58	2.26	0.37	2.5	0.74	1.89	0.15
GM-CSF	1.38	0.57	1.34	0.54	1.33	0.4	1.25	0.3
IL-1α	11.35	4.36	5.07	3.84	5.85	3.94	6.19	2.12
IL-1β	1.55	0.75	1.21	0.12	1.14	0.05	1.24	0.15
IL-2	1.77	0.69	1.39	0.19	1.67	0.69	1.23	0.18
IL-3	1.13	0.07	1.1	0.05	1.19	0.11	1.12	0.06
IL-4	1.06	0.04	1.1	0.03	1.09	0.02	1.08	0.02
IL-5	1.26	0.12	1.33	0.11	1.24	0.09	1.39	0.11
IL-6	1.19	0.15	1.12	0.05	1.14	0.07	1.19	0.19
IL-9	10.61	4.19	10.26	3.85	6.2	3.58	9.4	1.04
IP-10	3.8	2.06	3.5	1.02	4.62	3.4	3.44	0.98
KC	3.72	2.43	4.43	1.47	3.72	1.37	3.89	0.87
MCP-1	5.76	8.17	3.37	2.6	2.44	2.76	4.41	3.24
MIG	2.03	0.5	6.12	6.63	5.46	2.23	5.3	2.83
MIP-1α	5.28	3.43	3.94	1.53	4.47	3.38	5.65	3.46
MIP-2	14.16	5.98	17.75	4.17	19.45	7.01	19.99	2.37
TNFα	1.22	0.17	1.14	0.03	1.21	0.17	1.14	0.06
VEGF	7.76	1.06	7.86	1.93	6.27	1.07	6.67	1.23
TGF-β1	30.11	7.99	31.79	7.3	27.02	11.78	35.05	2.45
TGF-β2	18.78	4.3	14.93	4.42	18.19	9.88	18.44	4.28
TGF-β3	0.35	0.39	0.52	0.48	0.46	0.46	0.48	0.14
MMP-2	4815.46	2797.2	3559.44	1439.87	7070.31	5293.86	2549.08	1247.73
MMP-3	1942.97	937.28	2153.38	770.54	3341.66	2499.68	1645.06	659.54
MMP-8	3032.76	433.91	3566.74	640.66	5149.93	3568.21	3662.5	680.06
MMP-12	29.77	12.65	42.57	28.6	62.24	58.32	24.71	15.57
proMMP-9	208.2	114.38	152.91	46.7	254.31	215.11	157.32	126.73
IFNγ	OOR							
IL-7	OOR							
IL-10	OOR							
IL-12p40	OOR							
IL-12p70	OOR							
IL-13	OOR							
IL-15	OOR							
IL-17	OOR							
LIF	OOR							
LIX	OOR							
M-CSF	OOR							
RANTES	OOR							
MIP-1β	OOR							

OOR-Out of Range

Supplementary Table 4: Percentage DNAm of genes in Igf1 axis and PAH metabolism pathway.

	Control		HFD	
Genes	Mean % DNAm	SD	Mean % DNAm	SD
Males				
Igfbp1	41.31	2.45	42.63	1.56
Igfbp3	5.19	1.82	4.23	0.95
Gstm1	3.44	0.53	3.17	0.54
Ephx1	33.34	0.89	32.67	2.31
Gstt1	43.52	0.72	43.58	1.49
Gstp1	72.1	2.23	69.86	2.95
Cyp1a1	77.28	3.06	75.86	3.51
Ir	28.65	2.65	28.5	2.99
Ahrr pos1	70.56	2.16	70.47	1.31
Ahrr pos2	88.95	1.51	88.24	0.82
Ahr	43.19	2.55	41.6	0.24
Females				
Igfbp1	41.78	1.81	43.42	1.09
Igfbp3	5.13	0.58	4.22	0.3
Gstm1	3.4	0.87	3.12	0.14
Ephx1	33.57	4.03	30.96	4.37
Gstt1	42.68	2.46	42.47	1.85
Gstp1	70.11	2.66	69.22	2.04
Cyp1a1	76.79	2.1	75.11	0.78
Ir	27.34	3.49	26.85	2.02
Ahrr pos1	69.11	1.09	68.93	3.38
Ahrr pos2	86.63	1.04	86.98	2.14
Ahr	42.13	2.08	43.23	1.35

Supplementary Table 5: Log2 fold changes of gene abundance if genes in Igf1 axis and PAH metabolism pathway. The red highlighted ones indicate significant differences found, based on two-way ANOVA.

	Control	**Control-CS**	**HFD**	**HFD-CS**
Genes	Log2 FC	Log2 FC	Log2 FC	Log2 FC
Males				
Cyp1a1	0.15	2.76	1.19	2.81
Igf1	0.02	-0.22	-0.39	-0.31
Gstm1	0.01	0.37	0.2	0.34
Ahr	-0.01	0.17	0.27	-0.09
Ahrr	-0.01	0.34	0.27	0.43
Igfr1	0.02	0.64	0.17	0.63
Igfbp3	0.02	-1.28	-1.16	-0.47
Ir	0.01	-0.14	-0.05	0.17
Gstp1	0.01	0.1	0.04	0.64
Ephx1	0.01	-0.61	-0.17	0.59
Gstt1	0.01	-1.7	-0.31	0.41
Females				
Cyp1a1	-0.2	1.16	0.22	2.22
Igf1	-0.03	-0.5	-0.48	-0.17
Gstm1	0.02	0.19	-0.25	0.12
Ahr	-0.12	-0.34	-0.28	-0.24
Ahrr	-0.12	-0.54	-0.47	-0.06
Igfr1	-0.24	-0.73	-1.46	-1.57
Igfbp3	0.04	0.24	0.25	-0.08
Ir	-0.05	-0.1	-0.08	0.14
Gstp1	0.03	-0.06	-0.3	0.03
Ephx1	-0.07	-0.14	-0.83	0.47
Gstt1	-0.01	-0.26	-0.47	-0.1

References

Adgent, M. A., Gebretsadik, T., Reedus, J., Graves, C., Garrison, E., Bush, N., Davis, R., LeWinn, K. Z., Tylavsky, F., & Carroll, K. N. (2021). Gestational diabetes and childhood asthma in a racially diverse US pregnancy cohort. *Pediatric Allergy and Immunology*, *32*(6), 1190–1196. https://doi.org/10.1111/pai.13523

Aerts, L., & Van Assche, F. A. (2006). Animal evidence for the transgenerational development of diabetes mellitus. *International Journal of Biochemistry and Cell Biology*, *38*(5–6), 894–903. https://doi.org/10.1016/j.biocel.2005.07.006

Agudelo, C. W., Samaha, G., & Garcia-Arcos, I. (2020). Alveolar lipids in pulmonary disease. A review. In *Lipids in Health and Disease* (Vol. 19, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12944-020-01278-8

Allen, J. L. (2019). Airway function throughout the lifespan: Pediatric origins of adult respiratory disease. *Pediatric Investigation*, *3*(4), 236–244. https://doi.org/10.1002/ped4.12165

Arora, S., Dev, K., Agarwal, B., Das, P., & Syed, M. A. (2018). Macrophages: Their role, activation and polarization in pulmonary diseases. In *Immunobiology* (Vol. 223, Issues 4–5, pp. 383–396). Elsevier GmbH. https://doi.org/10.1016/j.imbio.2017.11.001

Aspberg, S., Dahlquist, G., Kahan, T., & Källén, B. (2007). Is neonatal phototherapy associated with an increased risk for hospitalized childhood bronchial asthma? *Pediatric Allergy and Immunology*, *18*(4), 313–319. https://doi.org/10.1111/j.1399-3038.2006.00518.x

Aspberg, S., Dahlquist, G., Kahan, T., & Källén, B. (2010). Confirmed association between neonatal phototherapy or neonatal icterus and risk of childhood asthma. *Pediatric Allergy and Immunology*, *21*, e733–e739. https://doi.org/10.1111/j.1399-3038.2010.01038.x

Atar, H. Y., Baatz, J. E., & Ryan, R. M. (2021). Molecular mechanisms of maternal diabetes effects on fetal and neonatal surfactant. *Children*, *8*(4), 1–10. https://doi.org/10.3390/children8040281

Azad, M. B., Becker, A. B., & Kozyrskyj, A. L. (2013a). Association of maternal diabetes and child asthma. *Pediatric Pulmonology*, *48*(6), 545–552. https://doi.org/10.1002/ppul.22668

Azad, M. B., Becker, A. B., & Kozyrskyj, A. L. (2013b). Association of maternal diabetes and child asthma. *Pediatric Pulmonology*, *48*(6), 545–552. https://doi.org/10.1002/ppul.22668

Azad, M. B., Moyce, B. L., Guillemette, L., Pascoe, C. D., Wicklow, B., McGavock, J. M., Halayko, A. J., & Dolinsky, V. W. (2017). Diabetes in pregnancy and lung health in offspring: developmental origins of respiratory disease. *Paediatric Respiratory Reviews*, *21*, 19–26. https://doi.org/10.1016/j.prrv.2016.08.007

Baack, M. L., Forred, B. J., Larsen, T. D., Jensen, D. N., Wachal, A. L., Khan, M. A., & Vitiello, P. F. (2016a). Consequences of a maternal high-fat diet and late gestation diabetes on the developing rat lung. *PLoS ONE*, *11*(8). https://doi.org/10.1371/journal.pone.0160818

Baack, M. L., Forred, B. J., Larsen, T. D., Jensen, D. N., Wachal, A. L., Khan, M. A., & Vitiello, P. F. (2016b). Consequences of a maternal high-fat diet and late gestation diabetes on the developing rat lung. *PLoS ONE*, *11*(8), 1–21. https://doi.org/10.1371/journal.pone.0160818

Baek, K. J., Cho, J. Y., Rosenthal, P., Alexander, L. E. C., Nizet, V., & Broide, D. H. (2013). Hypoxia potentiates allergen induction of HIF-1α, chemokines, airway inflammation, TGF-β1, and airway remodeling in a mouse model. *Clinical Immunology*, *147*(1), 27–37. https://doi.org/10.1016/j.clim.2013.02.004

Bai, Y., Zhou, Q., Fang, Q., Song, L., & Chen, K. (2019). Inflammatory cytokines and T-lymphocyte subsets in serum and sputum in patients with bronchial asthma and chronic obstructive pulmonary disease. *Medical Science Monitor*, *25*, 2206–2210. https://doi.org/10.12659/MSM.913703

Balaji, V., & Seshiah, V. (2011). Management of Diabetes in Pregnancy. *Journal of the Association of Physicians of India*, *59*, 1–5.

Bardoel, B. W., Kenny, E. F., Sollberger, G., & Zychlinsky, A. (2014). The balancing act of neutrophils. In *Cell Host and Microbe* (Vol. 15, Issue 5, pp. 526–536). Cell Press. https://doi.org/10.1016/j.chom.2014.04.011

Barnes, P. J. (2008). The cytokine network in asthma and chronic obstructive pulmonary disease. In *Journal of Clinical Investigation* (Vol. 118, Issue 11, pp. 3546–3556). https://doi.org/10.1172/JCI36130

Barnes, P. J. (2009). The cytokine network in chronic obstructive pulmonary disease. In *American Journal of Respiratory Cell and Molecular Biology* (Vol. 41, Issue 6, pp. 631–638). https://doi.org/10.1165/rcmb.2009-0220TR

Barnes, P. J. (2014). Cellular and molecular mechanisms of chronic obstructive pulmonary disease. In *Clinics in Chest Medicine* (Vol. 35, Issue 1, pp. 71–86). https://doi.org/10.1016/j.ccm.2013.10.004

Barnes, P. J. (2022). Oxidative Stress in Chronic Obstructive Pulmonary Disease. In *Antioxidants* (Vol. 11, Issue 5). MDPI. https://doi.org/10.3390/antiox11050965

Barnes, P. J., Shapiro, S. D., & Pauwels, R. A. (2003). Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. In *European Respiratory Journal* (Vol. 22, Issue 4, pp. 672–688). European Respiratory Society. https://doi.org/10.1183/09031936.03.00040703

Bartal, M. (2005). COPD and tobacco smoke. In *Monaldi Arch Chest Dis* (Vol. 63, Issue 4).

Belgrave, D. C. M., Granell, R., Turner, S. W., Curtin, J. A., Buchan, I. E., Le Souëf, P. N., Simpson, A., Henderson, A. J., & Custovic, A. (2018). Lung function trajectories from pre-school age to adulthood and their associations with early life factors: a retrospective analysis of three population-based birth cohort studies. *The Lancet Respiratory Medicine*, *6*(7), 526–534. https://doi.org/10.1016/S2213-2600(18)30099-7

Beyer et al 2009. (n.d.).

Bird, Y., Lemstra, M., Rogers, M., & Moraros, J. (2015). The relationship between socioeconomic status/income and prevalence of diabetes and associated conditions: A cross-sectional population-based study in Saskatchewan, Canada. *International Journal for Equity in Health*, *14*(1). https://doi.org/10.1186/s12939-015-0237-0

Bisgaard, H., Nørgaard, S., Sevelsted, A., Chawes, B. L., Stokholm, J., Mortensen, E. L., Ulrik, C. S., & Bønnelykke, K. (2021). Asthma-like symptoms in young children increase the risk of COPD. *Journal of Allergy and Clinical Immunology*, *147*(2), 569-576.e9. https://doi.org/10.1016/j.jaci.2020.05.043

Borsari, L., Malagoli, C., Werler, M. M., Rothman, K. J., Malavolti, M., Rodolfi, R., De Girolamo, G., Nicolini, F., & Vinceti, M. (2018). Joint Effect of Maternal Tobacco Smoking and Pregestational Diabetes on Preterm Births and Congenital Anomalies: A Population-Based Study in Northern Italy. *Journal of Diabetes Research*, *2018*. https://doi.org/10.1155/2018/2782741

Bossé, Y., Lamontagne, M., Gaudreault, N., Racine, C., Levesque, M. H., Smith, B. M., Auger, D., Clemenceau, A., Paré, M. È., Laviolette, L., Tremblay, V., Maranda, B., Morissette, M. C., & Maltais, F. (2019). Early-onset emphysema in a large French-Canadian family: a genetic investigation. *The Lancet Respiratory Medicine*, *7*(5), 427–436. https://doi.org/10.1016/S2213-2600(19)30056-6

Botelho, F. M., Bauer, C. M. T., Finch, D., Nikota, J. K., Zavitz, C. C. J., Kelly, A., Lambert, K. N., Piper, S., Foster, M. L., Goldring, J. J. P., Wedzicha, J. A., Bassett, J., Bramson, J., Iwakura, Y., Sleeman, M., Kolbeck, R., Coyle, A. J., Humbles, A. A., & Stämpfli, M. R. (2011). IL-1α/IL-1R1 expression in chronic obstructive pulmonary disease and mechanistic relevance to smoke-induced neutrophilia in mice. *PLoS ONE*, *6*(12). https://doi.org/10.1371/journal.pone.0028457

Breton, C. V., Byun, H. M., Wenten, M., Pan, F., Yang, A., & Gilliland, F. D. (2009). Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *American Journal of Respiratory and Critical Care Medicine*, *180*(5), 462–467. https://doi.org/10.1164/rccm.200901-0135OC

Broström, E. B., Akre, O., Katz-Salamon, M., Jaraj, D., & Kaijser, M. (2013). Obstructive pulmonary disease in old age among individuals born preterm. *European Journal of Epidemiology*, *28*(1), 79–85. https://doi.org/10.1007/s10654-013-9761-7

Brozyna, S., Ahern, J., Hodge, G., Nairn, J., Holmes, M., Reynolds, P. N., & Hodge, S. (2009). Chemotactic mediators of Th1 T-cell trafficking in smokers and COPD patients. *COPD: Journal of Chronic Obstructive Pulmonary Disease*, *6*(1), 4–16. https://doi.org/10.1080/15412550902724164

Bruzzaniti, S., Bocchino, M., Santopaolo, M., Calì, G., Stanziola, A. A., D'Amato, M., Esposito, A., Barra, E., Garziano, F., Micillo, T., Zuchegna, C., Romano, A., De Simone, S., Zuccarelli, B., Mottola, M., De Rosa, V., Porcellini, A., Perna, F., Matarese, G., & Galgani, M. (2019). An immunometabolic pathomechanism for chronic obstructive pulmonary disease. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(31), 15625–15634. https://doi.org/10.1073/pnas.1906303116

Buculei, I., Dobrin, M. E., Matei, D., Onu, I., Vicol, C., Cioroiu, I. B., Niculaua, M., Iordan, D. A., Cernomaz, A., & Trofor, A. C. (2022). Polycyclic Aromatic Hydrocarbons Induced by Smoking and Air Pollution: Correlation with Oxidative Stress in Chronic Obstructive Pulmonary Disease Patients. *Toxics*, *10*(11). https://doi.org/10.3390/toxics10110681

Bui, D. S., Lodge, C. J., Burgess, J. A., Lowe, A. J., Perret, J., Bui, M. Q., Bowatte, G., Gurrin, L., Johns, D. P., Thompson, B. R., Hamilton, G. S., Frith, P. A., James, A. L., Thomas, P. S., Jarvis, D., Svanes, C., Russell, M., Morrison, S. C., Feather, I., … Dharmage, S. C. (2018). Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. *The Lancet Respiratory Medicine*, *6*(7), 535–544. https://doi.org/10.1016/S2213-2600(18)30100-0

Cahill, K. M., Gartia, M. R., Sahu, S., Bergeron, S. R., Heffernan, L. M., Paulsen, D. B., Penn, A. L., & Noël, A. (2022). In utero exposure to electronic-cigarette aerosols decreases lung fibrillar collagen content, increases Newtonian resistance and induces sex-specific molecular signatures in neonatal mice. *Toxicological Research*, *38*(2), 205–224. https://doi.org/10.1007/s43188-021-00103-3

Carlo Massaro, G. DE, Massaro, D., Chan, W., Clerch, L. B., Ghyselinck, N., Chambon, P., S Chandraratna, R. A., De Carlo, G., Chan, W.-Y., & S Chandraratna Retinoic, R. A. (2000). *Retinoic acid receptor-: an endogenous inhibitor of the perinatal formation of pulmonary alveoli*. http://physiolgenomics.physiology.org

Carrapato, M. R. G. (2003). The offspring of gestational diabetes. *J. Perinat. Med.*, *31*, 5–11.

Chan, J. Y. C., Stern, D. A., Guerra, S., Wright, A. L., Morgan, W. J., & Martinez, F. D. (2015). Pneumonia in childhood and impaired lung function in adults: A longitudinal study. *Pediatrics*, *135*(4), 607–616. https://doi.org/10.1542/peds.2014-3060

Chen, C. H., Chan, C. C., Chen, B. Y., Cheng, T. J., & Leon Guo, Y. (2015). Effects of particulate air pollution and ozone on lung function in non-asthmatic children. *Environmental Research*, *137*, 40–48. https://doi.org/10.1016/j.envres.2014.11.021

Choi, W., Moon, J. H., Choi, H., Lee, H., Kim, H. K., Kang, H. C., & Cho, N. H. (2024). Trajectory of lung function in diabetic adults: A 16-year follow-up study of community-based prospective cohorts. *Respirology*, *29*(5), 413–420. https://doi.org/10.1111/resp.14658

Churg, A., Wang, R. D., Tai, H., Wang, X., Xie, C., & Wright, J. L. (2004). Tumor necrosis factor-α drives 70% of cigarette smoke-induced emphysema in the mouse. *American Journal of Respiratory and Critical Care Medicine*, *170*(5), 492–498. https://doi.org/10.1164/rccm.200404-511OC

Churg, A., Zhou, S., & Wright, J. L. (2012). Matrix metalloproteinases in COPD. In *European Respiratory Journal* (Vol. 39, Issue 1, pp. 197–209). https://doi.org/10.1183/09031936.00121611

Clair, C., Meigs, J. B., & Rigotti, N. A. (2013). Smoking behavior among US adults with diabetes or impaired fasting glucose. *American Journal of Medicine*, *126*(6), 541.e15-541.e18. https://doi.org/10.1016/j.amjmed.2012.11.029

Clausen, T. D., Mathiesen, E. R., Hansen, T., Pedersen, O., Jensen, D. M., Lauenborg, J., Schmidt, L., & Damm, P. (2009). Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. *Journal of Clinical Endocrinology and Metabolism*, *94*(7), 2464–2470. https://doi.org/10.1210/jc.2009-0305

Costa, C., Rufino, R., Traves, S. L., Lapa E Silva, J. R., Barnes, P. J., & Donnelly, L. E. (2008). CXCR3 and CCR5 chemokines in induced sputum from patients with COPD. *Chest*, *133*(1), 26–33. https://doi.org/10.1378/chest.07-0393

Culpitt, S. V., Rogers, D. F., Traves, S. L., Barnes, P. J., & Donnelly, L. E. (2005). Sputum matrix metalloproteases: Comparison between chronic obstructive pulmonary disease and asthma. *Respiratory Medicine*, *99*(6), 703–710. https://doi.org/10.1016/j.rmed.2004.10.022

Czovek, D. (2019). Pulmonary Function Tests in Infants and Children. *Kendig's Disorders of the Respiratory Tract in Children*, 174-211.e4. https://doi.org/10.1016/B978-0-323-44887-1.00011-0

Dabelea, D. (2007). The predisposition to obesity and diabetes in offspring of diabetic mothers. *Diabetes Care*, *30*(SUPPL. 2), 169–173. https://doi.org/10.2337/dc07-s211

Dai, X., Bui, D. S., Perret, J. L., Lowe, A. J., Frith, P. A., Bowatte, G., Thomas, P. S., Giles, G. G., Hamilton, G. S., Tsimiklis, H., Hui, J., Burgess, J., Win, A. K., Abramson, M. J., Walters, E. H., Dharmage, S. C., & Lodge, C. J. (2021). Exposure to household air pollution over 10 years is related to asthma and lung function decline. *European Respiratory Journal*, *57*(1). https://doi.org/10.1183/13993003.00602-2020

Dehmel, S., Nathan, P., Bartel, S., El-Merhie, N., Scherb, H., Milger, K., John-Schuster, G., Yildirim, A. O., Hylkema, M., Irmler, M., Beckers, J., Schaub, B., Eickelberg, O., & Krauss-Etschmann, S. (2018). Intrauterine smoke exposure deregulates lung function, pulmonary transcriptomes, and in particular insulin-like growth factor (IGF)-1 in a sex-specific manner. *Scientific Reports*, *8*(7547), 1–12. https://doi.org/10.1038/s41598-018-25762-5

Den Dekker, H. T., Sonnenschein-Van Der Voort, A. M. M., De Jongste, J. C., Anessi-Maesano, I., Arshad, S. H., Barros, H., Beardsmore, C. S., Bisgaard, H., Phar, S. C., Craig, L., Devereux, G., Van Der Ent, C. K., Esplugues, A., Fantini, M. P., Flexeder, C., Frey, U., Forastiere, F., Gehring, U., Gori, D., … Duijts, L. (2016). Early growth characteristics and the risk of reduced lung function and asthma: A meta-analysis of 25,000 children. *Journal of Allergy and Clinical Immunology*, *137*(4), 1026–1035. https://doi.org/10.1016/j.jaci.2015.08.050

Deolmi, M., Decarolis, N. M., Motta, M., Makrinioti, H., Fainardi, V., Pisi, G., & Esposito, S. (2023a). Early Origins of Chronic Obstructive Pulmonary Disease: Prenatal and Early Life Risk Factors. *International Journal of Environmental Research and Public Health*, *20*(3), 2294. https://doi.org/10.3390/ijerph20032294

Deolmi, M., Decarolis, N. M., Motta, M., Makrinioti, H., Fainardi, V., Pisi, G., & Esposito, S. (2023b). Early Origins of Chronic Obstructive Pulmonary Disease: Prenatal and Early

Life Risk Factors. In *International Journal of Environmental Research and Public Health* (Vol. 20, Issue 3). MDPI. https://doi.org/10.3390/ijerph20032294

Di Stefano, A., Capelli, A., & Donner, C. F. (2004). Role of interleukin-8 in the pathogenesis and treatment of COPD. In *Chest* (Vol. 126, Issue 3, pp. 676–678). American College of Chest Physicians. https://doi.org/10.1378/chest.126.3.676

Di Stefano, A., Caramori, G., Gnemmi, I., Contoli, M., Vicari, C., Capelli, A., Magno, F., D'Anna, S. E., Zanini, A., Brun, P., Casolari, P., Chung, K. F., Barnes, P. J., Papi, A., Adcock, I., & Balbi, B. (2009). T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. *Clinical and Experimental Immunology*, *157*(2), 316–324. https://doi.org/10.1111/j.1365-2249.2009.03965.x

Ding, Z., Wang, K., Li, J., Tan, Q., Tan, W., & Guo, G. (2019). Association between glutathione S-transferase gene M1 and T1 polymorphisms and chronic obstructive pulmonary disease risk: A meta-analysis. In *Clinical Genetics* (Vol. 95, Issue 1, pp. 53–62). Blackwell Publishing Ltd. https://doi.org/10.1111/cge.13373

Donnelly, L. E., & Barnes, P. J. (2006). Chemokine receptors as therapeutic targets in chronic obstructive pulmonary disease. In *Trends in Pharmacological Sciences* (Vol. 27, Issue 10, pp. 546–553). https://doi.org/10.1016/j.tips.2006.08.001

Doyle, L. W., Irving, L., Haikerwal, A., Lee, K., Ranganathan, S., & Cheong, J. (2019). Airway obstruction in young adults born extremely preterm or extremely low birth weight in the postsurfactant era. *Thorax*, *74*(12), 1147–1153. https://doi.org/10.1136/thoraxjnl-2019-213757

Drummond, D., Baravalle-Einaudi, M., Lezmi, G., Vibhushan, S., Franco-Montoya, M. L., Hadchouel, A., Boczkowski, J., & Delacourt, C. (2017). Combined effects of in utero and adolescent tobacco smoke exposure on lung function in C57BI/6J mice. *Environmental Health Perspectives*, *125*(3), 392–399. https://doi.org/10.1289/EHP54

Duan, P., Wang, Y., Lin, R., Zeng, Y., Chen, C., Yang, L., Yue, M., Zhong, S., Wang, Y., & Zhang, Q. (2021). Impact of early life exposures on COPD in adulthood: A systematic review and meta-analysis. *Respirology*, *26*(12), 1131–1151. https://doi.org/10.1111/resp.14144

Dumas, O., Arroyo, A. C., Faridi, M. K., James, K., Hsu, S., Powe, C., & Camargo, C. A. (2022). Cohort Study of Maternal Gestational Weight Gain, Gestational Diabetes, and Childhood Asthma. *Nutrients*, *14*(5188), 1–11. https://doi.org/10.3390/nu14235188

Edwards, C. A., Osman, L. M., Godden, D. J., Campbell, D. M., & Douglas, J. G. (2003). Relationship between birth weight and adult lung function: Controlling for maternal factors. *Thorax*, *58*(12), 1061–1065. https://doi.org/10.1136/thorax.58.12.1061

Eltboli, O., & Brightling, C. E. (2013). Eosinophils as diagnostic tools in chronic lung disease. In *Expert Review of Respiratory Medicine* (Vol. 7, Issue 1, pp. 33–42). Expert Reviews Ltd. https://doi.org/10.1586/ers.12.81

Feghali-Bostwick, C. A., Gadgil, A. S., Otterbein, L. E., Pilewski, J. M., Stoner, M. W., Csizmadia, E., Zhang, Y., Sciurba, F. C., & Duncan, S. R. (2008). Autoantibodies in

patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *177*(2), 156–163. https://doi.org/10.1164/rccm.200701-014OC

Finer, S., Mathews, C., Lowe, R., Smart, M., Hillman, S., Foo, L., Sinha, A., Williams, D., Rakyan, V. K., & Hitman, G. A. (2014). Maternal gestational diabetes is associated with genome-wide DNA methylation variation in placenta and cord blood of exposed offspring. *Human Molecular Genetics*, *24*(11), 3021–3029. https://doi.org/10.1093/hmg/ddv013

Fullerton, D. G., Suseno, ‡ A, Semple, † S, Kalambo, F., Malamba, R., White, S., Jack, S., Calverley, P. M., Gordon, S. B., & Fullerton, D. (2011). Wood smoke exposure, poverty and impaired lung function in Malawian adults. In *INT J TUBERC LUNG DIS* (Vol. 15, Issue 3).

Gillman, M. W. (2005). Developmental Origins of Health and Disease. *N Engl J Med*, *353*(17), 1848–1850.

Gläser, S., Friedrich, N., Ewert, R., Schäper, C., Nauck, M., Dörr, M., Völzke, H., Felix, S. B., Krebs, A., Wallaschofski, H., & Koch, B. (2009). Association between serum insulin-like growth factor (IGF) I and IGF binding protein-3 and lung function. *Journal of Clinical Endocrinology and Metabolism*, *94*(7), 2452–2458. https://doi.org/10.1210/jc.2008-2662

Goedtke, L., Sprenger, H., Hofmann, U., Schmidt, F. F., Hammer, H. S., Zanger, U. M., Poetz, O., Seidel, A., Braeuning, A., & Hessel-Pras, S. (2021). Polycyclic aromatic hydrocarbons activate the aryl hydrocarbon receptor and the constitutive androstane receptor to regulate xenobiotic metabolism in human liver cells. *International Journal of Molecular Sciences*, *22*(1), 1–17. https://doi.org/10.3390/ijms22010372

Gompertz, S., O9brien, C., Bayley, D. L., Hill, S. L., & Stockley, R. A. (2001). Changes in bronchial inflammation during acute exacerbations of chronic bronchitis. In *Eur Respir J* (Vol. 17).

González Corona, C., & Parchem, R. J. (2022). Maternal diabetes negatively impacts fetal health. *Open Biology*, *12*(9). https://doi.org/10.1098/rsob.220135

Gooptu, B., Ekeowa, U. I., & Lomas, D. A. (2008). Mechanisms of emphysema in α1-antitrypsin deficiency: Molecular and cellular insights. In *European Respiratory Journal* (Vol. 34, Issue 2, pp. 475–488). https://doi.org/10.1183/09031936.00096508

Gosman, M. M. E., Willemse, B. W. M., Jansen, D. F., Lapperre, T. S., van Schadewijk, A., Hiemstra, P. S., Postma, D. S., Timens, W., Kerstjens, H. A. M., Kauffman, H. F., de Reus, D., Boezen, H. M., van der Meulen, J. M., Vonk, J., Barentsen, M. D. W., Zeinstra-Smit, M., Luteijn, A. J., van der Molen, T., ter Veen, G., … Mauad, T. (2006). Increased number of B-cells in bronchial biopsies in COPD. *European Respiratory Journal*, *27*(1), 60–64. https://doi.org/10.1183/09031936.06.00007005

Grandi, C., Tapia, J. L., & Cardoso, V. C. (2015). Impact of maternal diabetes mellitus on mortality and morbidity of very low birth weight infants: A multicenter Latin America study. *Jornal de Pediatria*, *91*(3), 234–241. https://doi.org/10.1016/j.jped.2014.08.007

Green, C. J., Holly, J. M., Bolton, C. E., Bayer, A., Ebrahim, S., Gallacher, J., & Ben-Shlomo, Y. (2014). Original Article Role of IGF-I, IGF-II and IGFBP-3 in lung function of males: the Caerphilly Prospective Study. In *Int J Mol Epidemiol Genet* (Vol. 5, Issue 2). www.ijmeg.org

Griffiths, P. S., Walton, C., Samsell, L., Perez, M. K., & Piedimonte, G. (2016). Maternal high-fat hypercaloric diet during pregnancy results in persistent metabolic and respiratory abnormalities in offspring. *Pediatric Research*, *79*(2), 278–286. https://doi.org/10.1038/pr.2015.226

Guerra, S., Stern, D. A., Zhou, M., Sherrill, D. L., Wright, A. L., Morgan, W. J., & Martinez, F. D. (2013). Combined effects of parental and active smoking on early lung function deficits: A prospective study from birth to age 26 years. *Thorax*, *68*(11), 1021–1028. https://doi.org/10.1136/thoraxjnl-2013-203538

Haag, M., Leusink-Muis, T., Le Bouquin, R., Nijkamp, F. P., Lugnier, A., Frossard, N., Folkerts, G., & Pons, F. (2002). Increased expression and decreased activity of cytochrome P450 1A1 in a murine model of toluene diisocyanate-induced asthma. *Archives of Toxicology*, *76*(11), 621–627. https://doi.org/10.1007/s00204-002-0393-z

Halpin, D. M. G., Celli, B. R., Criner, G. J., Frith, P., López Varela, M. V., Salvi, S., Vogelmeier, C. F., Chen, R., Mortimer, K., Montes de Oca, M., Aisanov, Z., Obaseki, D., Decker, R., & Agusti, A. (2019). The GOLD Summit on chronic obstructive pulmonary disease in low- And middle-income countries. *International Journal of Tuberculosis and Lung Disease*, *23*(11), 1131–1141. https://doi.org/10.5588/ijtld.19.0397

Hami, J., Sadr-Nabavi, A., Sankian, M., Balali-Mood, M., & Haghir, H. (2013). The effects of maternal diabetes on expression of insulin-like growth factor-1 and insulin receptors in male developing rat hippocampus. *Brain Structure and Function*, *218*(1), 73–84. https://doi.org/10.1007/s00429-011-0377-y

Harju, M., Keski-Nisula, L., Georgiadis, L., & Heinonen, S. (2016a). Parental smoking and cessation during pregnancy and the risk of childhood asthma. *BMC Public Health*, *16*(428), 1–7. https://doi.org/10.1186/s12889-016-3029-6

Harju, M., Keski-Nisula, L., Georgiadis, L., & Heinonen, S. (2016b). Parental smoking and cessation during pregnancy and the risk of childhood asthma. *BMC Public Health*, *16*(1). https://doi.org/10.1186/s12889-016-3029-6

Hedderson, M. M., Ferrara, A., & Sacks, D. A. (2003). Gestational diabetes mellitus and lesser degrees of pregnancy hyperglycemia: Association with increased risk of spontaneous preterm birth. *Obstetrics and Gynecology*, *102*(4), 850–856. https://doi.org/10.1016/S0029-7844(03)00661-6

Hendrix, A. Y., & Kheradmand, F. (2017). The Role of Matrix Metalloproteinases in Development, Repair, and Destruction of the Lungs. In *Progress in Molecular Biology and Translational Science* (Vol. 148, pp. 1–29). Elsevier B.V. https://doi.org/10.1016/bs.pmbts.2017.04.004

Henrot, P., Prevel, R., Berger, P., & Dupin, I. (2019). Chemokines in COPD: From implication to therapeutic use. In *International Journal of Molecular Sciences* (Vol. 20, Issue 11). MDPI AG. https://doi.org/10.3390/ijms20112785

Hersh, C. P., Silverman, E. K., Gascon, J., Bhattacharya, S., Klanderman, B. J., Litonjua, A. A., Lefebvre, V., Sparrow, D., Reilly, J. J., Anderson, W. H., Lomas, D. A., & Mariani, T. J. (2011). SOX5 is a candidate gene for chronic obstructive pulmonary disease susceptibility and is necessary for lung development. *American Journal of Respiratory and Critical Care Medicine*, *183*(11), 1482–1489. https://doi.org/10.1164/rccm.201010-1751OC

Hervier, B., Russick, J., Cremer, I., & Vieillard, V. (2019). NK cells in the human lungs. In *Frontiers in Immunology* (Vol. 10, Issue JUN). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2019.01263

Heyob, K. M., Mieth, S., Sugar, S. S., Graf, A. E., Lallier, S. W., Britt, R. D., & Rogers, L. K. (2019). Maternal high-fat diet alters lung development and function in the offspring. *Am J Physiol Lung Cell Mol Physiol*, *317*, 167–174. https://doi.org/10.1152/aj

Higenbottam, T., Feyeraband, C., & Clark, T. J. H. (1980). Cigarette smoke inhalation and the acute airway response. In *Thorax* (Vol. 35).

Hirota, N., & Martin, J. G. (2013). Mechanisms of airway remodeling. *Chest*, *144*(3), 1026–1032. https://doi.org/10.1378/chest.12-3073

Hodge, G., Mukaro, V., Holmes, M., Reynolds, P. N., & Hodge, S. (2013). Enhanced cytotoxic function of natural killer and natural killer T-like cells associated with decreased CD94 (Kp43) in the chronic obstructive pulmonary disease airway. *Respirology*, *18*(2), 369–376. https://doi.org/10.1111/j.1440-1843.2012.02287.x

Hodge, G., Nairn, J., Holmes, M., Reynolds, P. N., & Hodge, S. (2007). Increased intracellular T helper 1 proinflammatory cytokine production in peripheral blood, bronchoalveolar lavage and intraepithelial T cells of COPD subjects. *Clinical and Experimental Immunology*, *150*(1), 22–29. https://doi.org/10.1111/j.1365-2249.2007.03451.x

Hoenderdos, K., & Condliffe, A. (2013). The neutrophil in chronic obstructive pulmonary disease: Too little, too late or too much, too soon? In *American Journal of Respiratory Cell and Molecular Biology* (Vol. 48, Issue 5, pp. 531–539). https://doi.org/10.1165/rcmb.2012-0492TR

Hogg, J. C., Chu, F., Utokaparch, S., Woods, R., Mark Elliott, W., Buzatu, L., Cherniack, R. M., Rogers, R. M., Sciurba, F. C., Coxson, H. O., & Paré, P. D. (2004). The Nature of Small-Airway Obstruction in Chronic Obstructive Pulmonary Disease. In *n engl j med* (Vol. 26). www.nejm.org

Hogg, J. C., & Timens, W. (2009). The pathology of chronic obstructive pulmonary disease. In *Annual Review of Pathology: Mechanisms of Disease* (Vol. 4, pp. 435–459). https://doi.org/10.1146/annurev.pathol.4.110807.092145

Howe, C. G., Cox, B., Fore, R., Jungius, J., Kvist, T., Lent, S., Miles, H. E., Salas, L. A., Rifas-Shiman, S., Starling, A. P., Yousefi, P., Ladd-Acosta, C., Baccarelli, A., Binder, E. B., Chatzi, V. L., Czamara, D., Dabelea, D., DeMeo, D. L., Ghantous, A., … Breton, C. V.

(2020). Maternal gestational diabetes mellitus and newborn DNA Methylation: Findings from the pregnancy and childhood epigenetics consortium. *Diabetes Care*, *43*(1), 98–105. https://doi.org/10.2337/dc19-0524

Huang, B. K., Elicker, B. M., Henry, T. S., Kallianos, K. G., Hahn, L. D., Tang, M., Heng, F., Mcculloch, C. E., Bhakta, N. R., Majumdar, S., Choi, J., Denlinger, L. C., Fain, S. B., Hastie, A. T., Hoffman, E. A., Israel, E., Jarjour, N. N., Levy, B. D., Mauger, D. T., … Fahy, J. V. (2024). *C L I N I C A L M E D I C I N E Persistent mucus plugs in proximal airways are consequential for airflow limitation in asthma*. https://doi.org/10.1172/jci

Huang, X., Mu, X., Deng, L., Aili, F., Pu, E., Tang, T., & Kong, X. (2019). The etiologic origins for chronic obstructive pulmonary disease. In *International Journal of COPD* (Vol. 14, pp. 1139–1158). Dove Medical Press Ltd. https://doi.org/10.2147/COPD.S203215

Innocentia, F., Fabbri, A., Anichinib, R., Tucia, S., Pettinb, G., Vannuccia, F., De Giorgio, L. A., & Seghieri, G. (1994). Indications of reduced pulmonary function in type 1 (insulin-dependent) diabetes mellitus *. *Diabetes Research and Clinical Practice*, *25*, 161–168.

James Gauderman, W., Avol, E., Gilliland, F., Vora, H., Thomas, D., Berhane, K., McConnell, R., Kuenzli, N., Lurmann, F., Rappaport, E., Margolis, H., Bates, D., & Peters, J. (2004). The Effect of Air Pollution on Lung Development from 10 to 18 Years of Age. *The New England Journal of Medicine*, *351*(11), 1057–1067. www.nejm.org

Jones, L. L., Hashim, A., McKeever, T., Cook, D. G., Britton, J., & Leonardi-Bee, J. (2011). Parental and household smoking and the increased risk of bronchitis, bronchiolitis and other lower respiratory infections in infancy: Systematic review and meta-analysis. *Respiratory Research*, *12*. https://doi.org/10.1186/1465-9921-12-5

Jose, A. (2018). Mepolizumab for eosinophilic chronic obstructive pulmonary disease. In *Journal of Clinical Outcomes Management* (Vol. 25, Issue 1). Turner White Communications Inc. https://doi.org/10.1056/nejmoa1708208

Joubert, B. R., Felix, J. F., Yousefi, P., Bakulski, K. M., Just, A. C., Breton, C., Reese, S. E., Markunas, C. A., Richmond, R. C., Xu, C. J., Küpers, L. K., Oh, S. S., Hoyo, C., Gruzieva, O., Söderhäll, C., Salas, L. A., Baïz, N., Zhang, H., Lepeule, J., … London, S. J. (2016). DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. *American Journal of Human Genetics*, *98*(4), 680–696. https://doi.org/10.1016/j.ajhg.2016.02.019

Ju, C. R., Liu, W., & Chen, R. C. (2012). Serum surfactant protein D: Biomarker of chronic obstructive pulmonary disease. *Disease Markers*, *32*(5), 281–287. https://doi.org/10.3233/DMA-2011-0887

Kelsen, S. G., Aksoy, M. O., Georgy, M., Hershman, R., Ji, R., Li, X. X., Hurford, M., Solomides, C., Chatila, W., & Kim, V. (2009). Lymphoid follicle cells in chronic obstructive pulmonary disease overexpress the chemokine receptor CXCR3. *American Journal of Respiratory and Critical Care Medicine*, *179*(9), 799–805. https://doi.org/10.1164/rccm.200807-1089OC

Kim, H. J., Choi, M. G., Park, M. K., & Seo, Y. R. (2017). Predictive and Prognostic Biomarkers of Respiratory Diseases due to Particulate Matter Exposure. *Journal of Cancer Prevention*, *22*(1), 6–15. https://doi.org/10.15430/jcp.2017.22.1.6

Kim, J. H., Sherman, M. E., Curriero, F. C., Guengerich, F. P., Strickland, P. T., & Sutter, T. R. (2004). Expression of cytochromes P450 1A1 and 1B1 in human lung from smokers, non-smokers, and ex-smokers. *Toxicology and Applied Pharmacology*, *199*(3), 210–219. https://doi.org/10.1016/j.taap.2003.11.015

King, P. T. (2015). Inflammation in chronic obstructive pulmonary disease and its role in cardiovascular disease and lung cancer. *Clinical and Translational Medicine*, *4*(1). https://doi.org/10.1186/s40169-015-0068-z

Köck, K., Köck, F., Klein, K., Bancher-Todesca, D., & Helmer, H. (2010). Diabetes mellitus and the risk of preterm birth with regard to the risk of spontaneous preterm birth. *Journal of Maternal-Fetal and Neonatal Medicine*, *23*(9), 1004–1008. https://doi.org/10.3109/14767050903551392

Kong, L., Nilsson, I. A. K., Gissler, M., & Lavebratt, C. (2019). Associations of Maternal Diabetes and Body Mass Index with Offspring Birth Weight and Prematurity. *JAMA Pediatrics*, *173*(4), 371–378. https://doi.org/10.1001/jamapediatrics.2018.5541

Kotecha, S. J., Edwards, M. O., Watkins, W. J., Henderson, A. J., Paranjothy, S., Dunstan, F. D., & Kotecha, S. (2013). Effect of preterm birth on later FEV1: A systematic review and meta-analysis. *Thorax*, *68*(8), 760–766. https://doi.org/10.1136/thoraxjnl-2012-203079

Kotecha, S. J., Watkins, W. J., Paranjothy, S., Dunstan, F. D., Henderson, A. J., & Kotecha, S. (2012). Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. *Thorax*, *67*(1), 54–61. https://doi.org/10.1136/thoraxjnl-2011-200329

Krishnan, J. K., & Martinez, F. J. (2018). Lung function trajectories and chronic obstructive pulmonary disease: Current understanding and knowledge gaps. *Current Opinion in Pulmonary Medicine*, *24*(2), 124–129. https://doi.org/10.1097/MCP.0000000000000456

Kumar, R., Ouyang, F., Story, R. E., Pongracic, J. A., Hong, X., Wang, G., Pearson, C., Ortiz, K., Bauchner, H., & Wang, X. (2009). Gestational diabetes, atopic dermatitis, and allergen sensitization in early childhood. *The Journal of Allergy and Clinical Immunology*, *124*(5), 1031–1038. https://doi.org/https://doi.org/10.1016/j.jaci.2009.06.052

Kythreotis, P., Kokkini, A., Avgeropoulou, S., Hadjioannou, A., Anastasakou, E., Rasidakis, A., & Bakakos, P. (2009). Plasma leptin and insulin-like growth factor I levels during acute exacerbations of chronic obstructive pulmonary disease. *BMC Pulmonary Medicine*, *9*. https://doi.org/10.1186/1471-2466-9-11

Lange, P., Celli, B., Agustí, A., Boje Jensen, G., Divo, M., Faner, R., Guerra, S., Marott, J. L., Martinez, F. D., Martinez-Camblor, P., Meek, P., Owen, C. A., Petersen, H., Pinto-Plata, V., Schnohr, P., Sood, A., Soriano, J. B., Tesfaigzi, Y., & Vestbo, J. (2015). Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. *New England Journal of Medicine*, *373*(2), 111–122. https://doi.org/10.1056/nejmoa1411532

Lange, P., Groth, S., Kastrup, J., Mortensen, J., Appleyard, M., Nyboe, J., Jensen, G., & Schnohr, P. (1989). Diabetes mellitus, plasma glucose and lung function in a cross-

sectional population study Diabetes mellitus, plasma glucose and lung function in a cross-sectional pop-ulation study. *Eur Respir J*, *2*, 14–19.

Larcombe, A. N., Foong, R. E., Berry, L. J., Zosky, G. R., & Sly, P. D. (2011). In utero cigarette smoke exposure impairs somatic and lung growth in BALB/c mice. *European Respiratory Journal*, *38*(4), 932–938. https://doi.org/10.1183/09031936.00156910

Lawlor, D. A., Ebrahim, S., & Smith, G. D. (2004). Associations of measures of lung function with insulin resistance and Type 2 diabetes: Findings from the British Women's Heart and Health Study. *Diabetologia*, *47*(2), 195–203. https://doi.org/10.1007/s00125-003-1310-6

Lawlor, D. A., Ebrahim, S., & Smith, G. D. (2005). Association of birth weight with adult lung function: Findings from the British Women's Heart and Health Study and a meta-analysis. *Thorax*, *60*(10), 851–858. https://doi.org/10.1136/thx.2005.042408

Lee, A. G., Le Grand, B., Hsu, H. H. L., Chiu, Y. H. M., Brennan, K. J., Bose, S., Rosa, M. J., Brunst, K. J., Kloog, I., Wilson, A., Schwartz, J., Morgan, W., Coull, B. A., Wright, R. O., Baccarelli, A. A., & Wright, R. J. (2018). Prenatal fine particulate exposure associated with reduced childhood lung function and nasal epithelia GSTP1 hypermethylation: Sex-specific effects. *Respiratory Research*, *19*(1), 76. https://doi.org/10.1186/s12931-018-0774-3

Lee, J. T., Son, J. Y., & Cho, Y. S. (2007). The adverse effects of fine particle air pollution on respiratory function in the elderly. *Science of the Total Environment*, *385*(1–3), 28–36. https://doi.org/10.1016/j.scitotenv.2007.07.005

Lee, K. W. K., Richmond, R., Hu, P., French, L., Shin, J., Bourdon, C., Reischl, E., Waldenberger, M., Zeilinger, S., Gaunt, T., McArdle, W., Ring, S., Woodward, G., Bouchard, L., Gaudet, D., Smith, G. D., Relton, C., Paus, T., & Pausova, Z. (2015). Prenatal exposure to maternal cigarette smoking and DNA methylation: Epigenome-wide association in a discovery sample of adolescents and replication in an independent cohort at birth through 17 years of age. *Environmental Health Perspectives*, *123*(2), 193–199. https://doi.org/10.1289/ehp.1408614

Lee, S. H., Goswami, S., Grudo, A., Song, L. Z., Bandi, V., Goodnight-White, S., Green, L., Hacken-Bitar, J., Huh, J., Bakaeen, F., Coxson, H. O., Cogswell, S., Storness-Bliss, C., Corry, D. B., & Kheradmand, F. (2007). Antielastin autoimmunity in tobacco smoking-induced emphysema. *Nature Medicine*, *13*(5), 567–569. https://doi.org/10.1038/nm1583

Leung-Pineda, V., & Gronowski, A. M. (2010). Biomarker tests for fetal lung maturity. *Biomarkers in Medicine*, *4*(6), 849–857. https://doi.org/10.2217/bmm.10.109

Levine, D. H. (1985). Hyperinsulinemia and Decreased Surfactant in Fetal Rabbits. *Dev. Pharmacol. Ther*, *8*, 284–291.

Li, G., Xing, Y., Wang, G., Wu, Q., Ni, W., Jiao, N., Chen, W., Liu, Q., Gao, L., Chao, C., Li, M., Wang, H., & Xing, Q. (2023). Does recurrent gestational diabetes mellitus increase the risk of preterm birth? A population-based cohort study. *Diabetes Research and Clinical Practice*, *199*, 1–9. https://doi.org/10.1016/j.diabres.2023.110628

Liang, C., DeCourcy, K., & Prater, M. R. (2010). High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. *Metabolism: Clinical and Experimental*, *59*(7), 943–950. https://doi.org/10.1016/j.metabol.2009.10.015

Liu, W., Ju, C. R., Chen, R. C., & Liu, Z. G. (2014). Role of serum and induced sputum surfactant protein D in predicting the response to treatment in chronic obstructive pulmonary disease. *Experimental and Therapeutic Medicine*, *8*(4), 1313–1317. https://doi.org/10.3892/etm.2014.1865

Liyanage, V. R. B., Jarmasz, J. S., Murugeshan, N., Bigio, M. R. D., Rastegar, M., & Davie, J. R. (2014). DNA Modifications: Function and Applications in Normal and Disease States. In *Biology* (Vol. 3, Issue 4, pp. 670–723). MDPI AG. https://doi.org/10.3390/biology3040670

Lo Tam Loi, A. T., Hoonhorst, S. J. M., Franciosi, L., Bischoff, R., Hoffmann, R. F., Heijink, I., Van Oosterhout, A. J. M., Boezen, H. M., Timens, W., Postma, D. S., Lammers, J. W., Koenderman, L., & Ten Hacken, N. H. T. (2013). Acute and chronic inflammatory responses induced by smoking in individuals susceptible and non-susceptible to development of COPD: From specific disease phenotyping towards novel therapy. Protocol of a cross-sectional study. *BMJ Open*, *3*(2), 1–9. https://doi.org/10.1136/bmjopen-2012-002178

Lomas, D. A. (2016). Does protease-antiprotease imbalance explain Chronic obstructive pulmonary disease? *Annals of the American Thoracic Society*, *13*(supplement 2), S130–S137. https://doi.org/10.1513/AnnalsATS.201504-196KV

Lopez, A. D., Shibuya, K., Rao, C., Mathers, C. D., Hansell, A. L., Held, L. S., Schmid, V., & Buist, S. (2006). Chronic obstructive pulmonary disease: Current burden and future projections. *European Respiratory Journal*, *27*(2), 397–412. https://doi.org/10.1183/09031936.06.00025805

Lovasi, G. S., Roux, A. V. D., Hoffman, E. A., Kawut, S. M., Jacobs, D. R., & Barr, R. G. (2010). Association of environmental tobacco smoke exposure in childhood with early emphysema in adulthood among nonsmokers. *American Journal of Epidemiology*, *171*(1), 54–62. https://doi.org/10.1093/aje/kwp358

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., AlMazroa, M. A., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., Baddour, L. M., Barker-Collo, S., Bartels, D. H., … Murray, C. J. L. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, *380*(9859), 2095–2128. https://doi.org/10.1016/S0140-6736(12)61728-0

Lv, M. Y., Qiang, L. X., Wang, B. C., Zhang, Y. P., Li, Z. H., Li, X. S., Jin, L. L., & Jin, S. De. (2022). Complex Evaluation of Surfactant Protein A and D as Biomarkers for the Severity of COPD. *International Journal of COPD*, *17*, 1537–1552. https://doi.org/10.2147/COPD.S366988

Maeda, Y., Davé, V., & Whitsett, J. A. (2007). *Transcriptional Control of Lung Morphogenesis*. https://doi.org/10.1152/physrev.00028.2006.-The

Magnus, M. C., Henderson, J., Tilling, K., Howe, L. D., & Fraser, A. (2018). Independent and combined associations of maternal and own smoking with adult lung function and COPD. *International Journal of Epidemiology*, *47*(6), 1855–1864. https://doi.org/10.1093/ije/dyy221

Marie F. Robert, Raymond K. Neff, John P. Hubbell, H. William Taeusch, & Mary Ellen Avery. (1976). Association between maternal diabetes and the respiratory-distress syndrome in the newborn. *The New England Journal of Medicine*, *294*(7), 357–360.

Martinez, F. D. (2016). Early-Life Origins of Chronic Obstructive Pulmonary Disease. *New England Journal of Medicine*, *375*(9), 871–878. https://doi.org/10.1056/nejmra1603287

Martinez, M. P., Lin, J., Chow, T., Chung, J., Wang, X., & Xiang, A. H. (2020). Maternal Gestational Diabetes and Type 2 Diabetes During Pregnancy and Risk of Childhood Asthma in Offspring. *Journal of Pediatrics*, *219*, 173-179.e1. https://doi.org/10.1016/j.jpeds.2019.12.053

Matsumura, K., & Ito, S. (2020). Novel biomarker genes which distinguish between smokers and chronic obstructive pulmonary disease patients with machine learning approach. *BMC Pulmonary Medicine*, *20*(1). https://doi.org/10.1186/s12890-020-1062-9

Mayor, R. S., Finch, K. E., Zehr, J., Morselli, E., Neinast, M. D., Frank, A. P., Hahner, L. D., Wang, J., Rakheja, D., Palmer, B. F., Rosenfeld, C. R., Savani, R. C., & Clegg, D. J. (2015a). Maternal high-fat diet is associated with impaired fetal lung development. *Am J Physiol Lung Cell Mol Physiol*, *309*, 360–368. https://doi.org/10.1152/ajplung.00105.2015.-Maternal

Mayor, R. S., Finch, K. E., Zehr, J., Morselli, E., Neinast, M. D., Frank, A. P., Hahner, L. D., Wang, J., Rakheja, D., Palmer, B. F., Rosenfeld, C. R., Savani, R. C., & Clegg, D. J. (2015b). Maternal high-fat diet is associated with impaired fetal lung development. *Am J Physiol Lung Cell Mol Physiol*, *309*, 360–368. https://doi.org/10.1152/ajplung.00105.2015.-Maternal

McEvoy, C. T., Schilling, D., Clay, N., Jackson, K., Go, M. D., Spitale, P., Bunten, C., Leiva, M., Gonzales, D., Hollister-Smith, J., Durand, M., Frei, B., Buist, A. S., Peters, D., Morris, C. D., & Spindel, E. R. (2014). Vitamin C supplementation for pregnant smoking women and pulmonary function in their newborn infants: A randomized clinical trial. *JAMA*, *311*(20), 2074–2082. https://doi.org/10.1001/jama.2014.5217

McEvoy, C. T., Shorey-Kendrick, L. E., Milner, K., Harris, J., Vuylsteke, B., Cunningham, M., Tiller, C., Stewart, J., Schilling, D., Brownsberger, J., Titus, H., Macdonald, K. D., Gonzales, D., Vu, A., Park, B. S., Spindel, E. R., Morris, C. D., & Tepper, R. S. (2023). Effect of Vitamin C Supplementation for Pregnant Smokers on Offspring Airway Function and Wheeze at Age 5 Years: Follow-up of a Randomized Clinical Trial. *JAMA Pediatrics*, *177*(1), 16–24. https://doi.org/10.1001/jamapediatrics.2022.4401

McEvoy, C. T., & Spindel, E. R. (2017). Pulmonary Effects of Maternal Smoking on the Fetus and Child: Effects on Lung Development, Respiratory Morbidities, and Life Long Lung Health. In *Paediatric Respiratory Reviews* (Vol. 21, pp. 27–33). W.B. Saunders Ltd. https://doi.org/10.1016/j.prrv.2016.08.005

McGeachie, M. J., Yates, K. P., Zhou, X., Guo, F., Sternberg, A. L., Van Natta, M. L., Wise, R. A., Szefler, S. J., Sharma, S., Kho, A. T., Cho, M. H., Croteau-Chonka, D. C., Castaldi, P. J., Jain, G., Sanyal, A., Zhan, Y., Lajoie, B. R., Dekker, J., Stamatoyannopoulos, J., … Strunk, R. C. (2016). Patterns of Growth and Decline in Lung Function in Persistent Childhood Asthma. *New England Journal of Medicine*, *374*(19), 1842–1852. https://doi.org/10.1056/nejmoa1513737

McKeever, T. M., Weston, P. J., Hubbard, R., & Fogarty, A. (2005). Lung function and glucose metabolism: An analysis of data from the Third National Health and Nutrition Examination Survey. *American Journal of Epidemiology*, *161*(6), 546–556. https://doi.org/10.1093/aje/kwi076

Meghji, J., Mortimer, K., Agusti, A., Allwood, B. W., Asher, I., Bateman, E. D., Bissell, K., Bolton, C. E., Bush, A., Celli, B., Chiang, C. Y., Cruz, A. A., Dinh-Xuan, A. T., El Sony, A., Fong, K. M., Fujiwara, P. I., Gaga, M., Garcia-Marcos, L., Halpin, D. M. G., … Marks, G. B. (2021). Improving lung health in low-income and middle-income countries: from challenges to solutions. In *The Lancet* (Vol. 397, Issue 10277, pp. 928–940). Elsevier B.V. https://doi.org/10.1016/S0140-6736(21)00458-X

Mendy, A., Forno, E., Niyonsenga, T., & Gasana, J. (2018). Blood biomarkers as predictors of long-term mortality in COPD. *Clinical Respiratory Journal*, *12*(5), 1891–1899. https://doi.org/10.1111/crj.12752

Meyer, K. F., Krauss-Etschmann, S., Kooistra, W., Reinders-Luinge, M., Timens, W., Kobzik, L., Plösch, T., Hylkema, X. M. N., & Hylkema, M. N. (2017). Prenatal exposure to tobacco smoke sex dependently influences methylation and mRNA levels of the Igf axis in lungs of mouse offspring. *Am J Physiol Lung Cell Mol Physiol*, *312*, 542–555. https://doi.org/10.1152/ajplung.00271.2016.-Prenatal

Mihalovičová, L., Kunšteková, V., Miláček, D., Janko, J., Pastorek, M., Konečná, B., Gurecká, R., Rausová, Z., Uličná, O., Celec, P., & Šebeková, K. (2023). Severe gestational diabetes mellitus in lean dams is associated with low IL-1α levels and affects the growth of the juvenile mouse offspring. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-28903-7

Milad, N., Pineault, M., Lechasseur, A., Routhier, J., Beaulieu, M.-J., Aubin, S., & Morissette, M. C. (2021). Neutrophils and IL-1α Regulate Surfactant Homeostasis during Cigarette Smoking. *The Journal of Immunology*, *206*(8), 1923–1931. https://doi.org/10.4049/jimmunol.2001182

Milne, K. M., Mitchell, R. A., Ferguson, O. N., Hind, A. S., & Guenette, J. A. (2024). Sex-differences in COPD: from biological mechanisms to therapeutic considerations. In *Frontiers in Medicine* (Vol. 11). Frontiers Media SA. https://doi.org/10.3389/fmed.2024.1289259

Mollerup, S., Berge, G., Bæra, R., Skaug, V., Hewer, A., Phillips, D. H., Stangeland, L., & Haugen, A. (2006). Sex differences in risk of lung cancer: Expression of genes in the PAH bioactivation pathway in relation to smoking and bulky DNA adducts. *International Journal of Cancer*, *119*(4), 741–744. https://doi.org/10.1002/ijc.21891

Moore, L. E., Serrano-Lomelin, J., Rosychuk, R. J., Kozyrskyj, A. L., Chari, R., Crawford, S., Bakal, J., Hicks, A., Ducharme, F. M., & Ospina, M. B. (2023). Perinatal and early life factors and asthma control among preschoolers: A population-based retrospective cohort study. *BMJ Open Respiratory Research*, *10*(1), 1–9. https://doi.org/10.1136/bmjresp-2023-001928

Morales, E., Garcia-Esteban, R., De La Cruz, O. A., Basterrechea, M., Lertxundi, A., Martinez López de Dicastillo, M. D., Zabaleta, C., & Sunyer, J. (2015). Intrauterine and early postnatal exposure to outdoor air pollution and lung function at preschool age. *Thorax*, *70*(1), 64–73. https://doi.org/10.1136/thoraxjnl-2014-205413

Nakamura, H. (2011). Genetics of COPD. *Allergology International*, *60*(3), 253–258. https://doi.org/10.2332/allergolint.11-RAI-0326

Narang, I., Rosenthal, M., Cremonesini, D., Silverman, M., & Bush, A. (2008). Longitudinal evaluation of airway function 21 years after preterm birth. *American Journal of Respiratory and Critical Care Medicine*, *178*(1), 74–80. https://doi.org/10.1164/rccm.200705-701OC

Narendra, D. K., & Hanania, N. A. (2019). Targeting IL-5 in COPD. In *International Journal of COPD* (Vol. 14, pp. 1045–1051). Dove Medical Press Ltd. https://doi.org/10.2147/COPD.S155306

Nelson, C. R. M., Dzakpasu, S., Moore, A. M., Darling, E. K., Edwards, W., Murphy, P., Scott, H., Van Den Hof, M., & Ray, J. G. (2024). Diabetes mellitus in pregnancy across Canada. *BMC Pregnancy and Childbirth*, *24*(349), 1–11. https://doi.org/10.1186/s12884-024-06534-8

Nikota, J. K., Shen, P., Morissette, M. C., Fernandes, K., Roos, A., Chu, D. K., Barra, N. G., Iwakura, Y., Kolbeck, R., Humbles, A. A., & Stampfli, M. R. (2014). Cigarette Smoke Primes the Pulmonary Environment to IL-1α/CXCR-2–Dependent Nontypeable Haemophilus influenzae –Exacerbated Neutrophilia in Mice . *The Journal of Immunology*, *193*(6), 3134–3145. https://doi.org/10.4049/jimmunol.1302412

Nkadi, P. O., Merritt, T. A., & Pillers, D. A. M. (2009). An overview of pulmonary surfactant in the neonate: Genetics, metabolism, and the role of surfactant in health and disease. In *Molecular Genetics and Metabolism* (Vol. 97, Issue 2, pp. 95–101). https://doi.org/10.1016/j.ymgme.2009.01.015

Núñez, B., Sauleda, J., Antó, J. M., Julià, M. R., Orozco, M., Monsó, E., Noguera, A., Gómez, F. P., Garcia-Aymerich, J., & Agustí, A. (2011). Anti-tissue antibodies are related to lung function in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *183*(8), 1025–1031. https://doi.org/10.1164/rccm.201001-0029OC

Obeidat, M., Li, X., Burgess, S., Zhou, G., Fishbane, N., Hansel, N. N., Bossé, Y., Joubert, P., Hao, K., Nickle, D. C., van den Berge, M., Timens, W., Cho, M. H., Hobbs, B. D., de Jong, K., Boezen, M., Hung, R. J., Rafaels, N., Mathias, R., … Sin, D. D. (2017). Surfactant protein D is a causal risk factor for COPD: Results of Mendelian randomisation. *European Respiratory Journal*, *50*(5), 1–11. https://doi.org/10.1183/13993003.00657-2017

O'Donnell, R. A., Peebles, C., Ward, J. A., Daraker, A., Angco, G., Broberg, P., Pierrou, S., Lund, J., Holgate, S. T., Davies, D. E., Delany, D. J., Wilson, S. J., & Djukanovic, R. (2004). Relationship between peripheral airway dysfunction, airway obstruction, and neutrophilic inflammation in COPD. *Thorax*, *59*(10), 837–842. https://doi.org/10.1136/thx.2003.019349

Olynik, B. M., & Rastegar, M. (2012). The genetic and epigenetic journey of embryonic stem cells into mature neural cells. In *Frontiers in Genetics* (Vol. 3, Issue MAY). https://doi.org/10.3389/fgene.2012.00081

Omori, K., Iwamoto, H., Yamane, T., Nakashima, T., Haruta, Y., Hattori, N., Yokoyama, A., & Kohno, N. (2017). Clinically remitted childhood asthma is associated with airflow obstruction in middle-aged adults. *Respirology*, *22*(1), 86–92. https://doi.org/10.1111/resp.12860

Onuzulu, C. D., Lee, S., Basu, S., Comte, J., Hai, Y., Hizon, N., Chadha, S., Fauni, M. S., Kahnamoui, S., Xiang, B., Halayko, A. J., Dolinsky, V. W., Pascoe, C. D., & Jones, M. J. (2023a). Early-life exposure to cigarette smoke primes lung function and DNA methylation changes at Cyp1a1 upon exposure later in life. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *325*(5), L552–L567. https://doi.org/10.1152/ajplung.00192.2023

Onuzulu, C. D., Lee, S., Basu, S., Comte, J., Hai, Y., Hizon, N., Chadha, S., Fauni, M. S., Kahnamoui, S., Xiang, B., Halayko, A. J., Dolinsky, V. W., Pascoe, C. D., & Jones, M. J. (2023b). Early-life exposure to cigarette smoke primes lung function and DNA methylation changes at Cyp1a1 upon exposure later in life. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *325*(5), L552–L567. https://doi.org/10.1152/ajplung.00192.2023

Ostridge, K., Williams, N., Kim, V., Bennett, M., Harden, S., Welch, L., Bourne, S., Coombs, N. A., Elkington, P. T., Staples, K. J., & Wilkinson, T. M. A. (2016). Relationship between pulmonary matrix metalloproteinases and quantitative CT markers of small airways disease and emphysema in COPD. *Thorax*, *71*(2), 126–132. https://doi.org/10.1136/thoraxjnl-2015-207428

Park, J.-Y. K., Shigenaga, M. K., & Ames, B. N. (1996). Induction of cytochrome P4501A1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin or indolo(3,2-b)carbazole is associated with oxidative DNA damage. In *Biochemistry* (Vol. 93). https://www.pnas.org

Pascoe, C. D., Basu, S., Schwartz, J., Fonseca, M., Kahnamoui, S., Jha, A., Dolinsky, V., & Halayko, A. J. (2022). Maternal diabetes promotes offspring lung dysfunction and inflammation in a sex-dependent manner. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *322*(3), L373–L384. https://doi.org/10.1152/ajplung.00425.2021

Pereira, T. J., Fonseca, M. A., Campbell, K. E., Moyce, B. L., Cole, L. K., Hatch, G. M., Doucette, C. A., Klein, J., Aliani, M., & Dolinsky, V. W. (2015). Maternal obesity characterized by gestational diabetes increases the susceptibility of rat offspring to hepatic steatosis via a disrupted liver metabolome. *Journal of Physiology*, *593*(14), 3181–3197. https://doi.org/10.1113/JP270429

Pilewski, J. M., Liu, L., Henry, A. C., Knauer, A. V, & Feghali-Bostwick, C. A. (n.d.). Insulin-Like Growth Factor Binding Proteins 3 and 5 Are Overexpressed in Idiopathic Pulmonary Fibrosis and Contribute to Extracellular Matrix Deposition. In *Cell Cycle Molecules*.

Piper, J. M., Xenakis, E. M. J., & Langer, O. (1998). Delayed appearance of pulmonary maturation markers is associated with poor glucose control in diabetic1 pregnancies. *Journal of Maternal-Fetal and Neonatal Medicine*, *7*(3), 148–153. https://doi.org/10.3109/14767059809020431

Piyadasa, H., Altieri, A., Basu, S., Schwartz, J., Halayko, A. J., & Mookherjee, N. (2016). Biosignature for airway inflammation in a house dust mite-challenged murine model of allergic asthma. *Biology Open*, *5*(2), 112–121. https://doi.org/10.1242/bio.014464

Polverino, F., Cosio, B. G., Pons, J., Laucho-Contreras, M., Tejera, P., Iglesias, A., Rios, A., Jahn, A., Sauleda, J., Divo, M., Pinto-Plata, V., Sholl, L., Rosas, I. O., Agustí, A., Celli, B. R., & Owen, C. A. (2015). B cell-activating factor an orchestrator of lymphoid follicles in severe chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *192*(6), 695–705. https://doi.org/10.1164/rccm.201501-0107OC

Polverino, F., Seys, L. J. M., Bracke, K. R., & Owen, C. A. (2016). B cells in chronic obstructive pulmonary disease: moving to center stage. *Am J Physiol Lung Cell Mol Physiol*, *311*, 687–695. https://doi.org/10.1152/ajplung.00304.2016.-Chronic

Preda, A., Iliescu, D. G., Comănescu, A., Zorilă, G. L., Vladu, I. M., Forțofoiu, M. C., Țenea-Cojan, T. S., Preda, S. D., Diaconu, I. D., Moța, E., Gheorghe, I. O., & Moța, M. (2023a). Gestational Diabetes and Preterm Birth: What Do We Know? Our Experience and Mini-Review of the Literature. *Journal of Clinical Medicine*, *12*(14). https://doi.org/10.3390/jcm12144572

Preda, A., Iliescu, D. G., Comănescu, A., Zorilă, G. L., Vladu, I. M., Forțofoiu, M. C., Țenea-Cojan, T. S., Preda, S. D., Diaconu, I. D., Moța, E., Gheorghe, I. O., & Moța, M. (2023b). Gestational Diabetes and Preterm Birth: What Do We Know? Our Experience and Mini-Review of the Literature. *Journal of Clinical Medicine*, *12*(4572), 1–10. https://doi.org/10.3390/jcm12144572

Proskocil, B. J., Sekhon, H. S., Clark, J. A., Lupo, S. L., Jia, Y., Hull, W. M., Whitsett, J. A., Starcher, B. C., & Spindel, E. R. (2005). Vitamin C prevents the effects of prenatal nicotine on pulmonary function in newborn monkeys. *American Journal of Respiratory and Critical Care Medicine*, *171*(9), 1032–1039. https://doi.org/10.1164/rccm.200408-1029OC

PUNTARULO and CEDERBAUM 1998. (n.d.).

Qin, K., Xu, B., Pang, M., Wang, H., & Yu, B. (2022). The functions of CD4 T-helper lymphocytes in chronic obstructive pulmonary disease. In *Acta Biochimica et Biophysica Sinica* (Vol. 54, Issue 2, pp. 173–178). Science Press. https://doi.org/10.3724/abbs.2021009

Quinn, J. A., Munoz, F. M., Gonik, B., Frau, L., Cutland, C., Mallett-Moore, T., Kissou, A., Wittke, F., Das, M., Nunes, T., Pye, S., Watson, W., Ramos, A. M. A., Cordero, J. F., Huang, W. T., Kochhar, S., & Buttery, J. (2016). Preterm birth: Case definition &

guidelines for data collection, analysis, and presentation of immunisation safety data. *Vaccine*, *34*(49), 6047–6056. https://doi.org/10.1016/j.vaccine.2016.03.045

Ramin, N., Thieme, R., Fischer, S., Schindler, M., Schmidt, T., Fischer, B., & Navarrete Santos, A. (2010). Maternal diabetes impairs gastrulation and insulin and IGF-I receptor expression in rabbit blastocysts. *Endocrinology*, *151*(9), 4158–4167. https://doi.org/10.1210/en.2010-0187

Reddy, K. D., Lan, A., Boudewijn, I. M., Rathnayake, S. N. H., Koppelman, G. H., Aliee, H., Theis, F., Oliver, B. G., van den Berge, M., & Faiz, A. (2021). Current smoking alters gene expression and DNA methylation in the nasal epithelium of patients with asthma. *American Journal of Respiratory Cell and Molecular Biology*, *65*(4), 366–377. https://doi.org/10.1165/rcmb.2020-0553OC

Rennard, S. I. (2007). Inflammation in COPD: A link to systemic comorbidities. *European Respiratory Review*, *16*(105), 91–97. https://doi.org/10.1183/09059180.00010502

Ritzmann, F., & Beisswenger, C. (2021). Preclinical studies and the function of IL-17 cytokines in COPD. In *Annals of Anatomy* (Vol. 237). Elsevier GmbH. https://doi.org/10.1016/j.aanat.2021.151729

Rizzi, M., Sergi, M., Andreoli, A., Pecis, M., Bruschi, C., & Fanfulla, F. (2004). Environmental tobacco smoke may induce early lung damage in healthy male adolescents. *Chest*, *125*(4), 1387–1393. https://doi.org/10.1378/chest.125.4.1387

Ross, J. C., San, R., Estépar, J., Ash, S., Pistenmaa, C., Han, M., Bhatt, S. P., Bodduluri, S., Sparrow, D., Charbonnier, J.-P., Washko, G. R., & Diaz, A. A. (2024). Dysanapsis is differentially related to lung function trajectories with distinct structural and functional patterns in COPD and variable risk for adverse outcomes. *EClinicalMedicine*, *68*(102408), 1–12. https://doi.org/https://doi.org/10. 1016/j.eclinm.2023. 102408

Ruchat, S. M., Houde, A. A., Voisin, G., St-Pierre, J., Perron, P., Baillargeon, J. P., Gaudet, D., Hivert, M. F., Brisson, D., & Bouchard, L. (2013). Gestational diabetes mellitus epigenetically affects genes predominantly involved in metabolic diseases. *Epigenetics*, *8*(9), 935–943. https://doi.org/10.4161/epi.25578

Rusconi, F., Galassi, C., Forastiere, F., Bellasio, M., De Sario, M., Ciccone, G., Brunetti, L., Chellini, E., Corbo, G., La Grutta, S., Lombardi, E., Piffer, S., Talassi, F., Biggeri, A., Pearce, N., Mirabelli, D., Migliore, E., Berti, G., Cadum, E., … Rana, M. (2007). Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children. *American Journal of Respiratory and Critical Care Medicine*, *175*(1), 16–21. https://doi.org/10.1164/rccm.200512-1978OC

Sadler, N. C., Webb-Robertson, B. J. M., Clauss, T. R., Pounds, J. G., Corley, R., & Wright, A. T. (2018). High-Fat Diets Alter the Modulatory Effects of Xenobiotics on Cytochrome P450 Activities. *Chemical Research in Toxicology*, *31*(5), 308–318. https://doi.org/10.1021/acs.chemrestox.8b00008

Salvi, S. S., & Barnes, P. J. (2009). Chronic obstructive pulmonary disease in non-smokers. *Lancet (London, England)*, *374*(9691), 733–743. https://doi.org/https://doi.org/10.1016/S0140-6736(09)61303-9

Sauty, A., Dziejman, M., Taha, R. A., Iarossi, A. S., Neote, K., Garcia-Zepeda, E. A., Hamid, Q., & Luster, A. D. (1999). The T Cell-Specific CXC Chemokines IP-10, Mig, and I-TAC Are Expressed by Activated Human Bronchial Epithelial Cells. *The Journal of Immunology*, *162*(6), 3549–3558. https://doi.org/10.4049/jimmunol.162.6.3549

Sears, M. R., Greene, J. M., Willan, A. R., Wiecek, E. M., Taylor, D. R., Flannery, E. M., Cowan, J. O., Herbison, G. P., Silva, P. A., & Poulton, R. (2003). A Longitudinal, Population-Based, Cohort Study of Childhood Asthma Followed to Adulthood. *N Engl J Med*, *349*, 1414–1436. www.nejm.org

Selle, J., Dinger, K., Jentgen, V., Zanetti, D., Will, J., Georgomanolis, T., Vohlen, C., Wilke, R., Kojonazarov, B., Klymenko, O., Mohr, J., v. Koningsbruggen-Rietschel, S., Rhodes, C. J., Ulrich, A., Hirani, D., Nestler, T., Odenthal, M., Mahabir, E., Nayakanti, S., … Alejandre Alcazar, M. A. (2022). Maternal and perinatal obesity induce bronchial obstruction and pulmonary hypertension via IL-6-FoxO1-axis in later life. *Nature Communications*, *13*(4352), 1–21. https://doi.org/10.1038/s41467-022-31655-z

Seminar Introduction. (n.d.). www.thelancet.com

Shakoori, T. A., Sin, D. D., Ghafoor, F., Bashir, S., Nazim, S., & Bokhari, H. (2009). Serum surfactant protein D during acute exacerbations of chronic obstructive pulmonary disease. *Disease Markers*, *27*, 287–294. https://doi.org/10.3233/DMA-2009-0674

Shertzer, H. G., Nebert, D. W., Puga, A., Ary, M., Sonntag, D., Dixon, K., Robinson, L. J., Cianciolo, E., & Dalton, T. P. (1998). *Dioxin Causes a Sustained Oxidative Stress Response in the Mouse*.

Simpson, A., Maniatis, N., Jury, F., Cakebread, J. A., Lowe, L. A., Holgate, S. T., Woodcock, A., Oilier, W. E. R., Collins, A., Custovic, A., Holloway, J. W., & John, S. L. (2005). Polymorphisms in a disintegrin and metalloprotease 33 (ADAM33) predict impaired early-life lung function. *American Journal of Respiratory and Critical Care Medicine*, *172*(1), 55–60. https://doi.org/10.1164/rccm.200412-1708OC

Singh, D., Edwards, L., Tal-Singer, R., & Rennard, S. (2010). Open Access RESEARCH Sputum neutrophils as a biomarker in COPD: findings from the ECLIPSE study. In *Respiratory Research* (Vol. 11). http://respiratory-research.com/content/11/1/77

Słupecka-Ziemilska, M., Wychowański, P., & Puzianowska-Kuznicka, M. (2020). Gestational diabetes mellitus affects offspring's epigenome. Is there a way to reduce the negative consequences? In *Nutrients* (Vol. 12, Issue 9, pp. 1–14). MDPI AG. https://doi.org/10.3390/nu12092792

Smith, C. A. D., & Harrison, D. J. (1997). *Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema Early report* (Vol. 350).

Stănescu, D., Sanna, A., Veriter, C., Kostianev, S., Calcagni, P. G., Fabbri, L. M., & Maestrelli, P. (1996). Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax*, *51*(3), 267–271. https://doi.org/10.1136/thx.51.3.267

Stanhope, K. K., Joseph, N. T., Platner, M., Hutchison, C., Wen, S., Laboe, A., Labgold, K., Jamieson, D. J., & Boulet, S. L. (2021). Validation of ICD-10 Codes for Gestational and

Pregestational Diabetes During Pregnancy in a Large, Public Hospital. *Epidemiology*, *32*(2), 277–281. https://doi.org/10.1097/EDE.0000000000001311

Sukjamnong, S., Chan, Y. L., Zakarya, R., Saad, S., Sharma, P., Santiyanont, R., Chen, H., Brian, X., & Oliver, G. (2017). Effect of long-term maternal smoking on the offspring's lung health. *Am J Physiol Lung Cell Mol Physiol*, *313*, 416–423. https://doi.org/10.1152/ajplung.00134.2017.-Maternal

Sun, W., Kechris, K., Jacobson, S., Drummond, M. B., Hawkins, G. A., Yang, J., Chen, T. H., Quibrera, P. M., Anderson, W., Barr, R. G., Basta, P. V., Bleecker, E. R., Beaty, T., Casaburi, R., Castaldi, P., Cho, M. H., Comellas, A. P., Crapo, J. D., Criner, G., … Ruiz, M. E. (2016). Common Genetic Polymorphisms Influence Blood Biomarker Measurements in COPD. *PLoS Genetics*, *12*(8), 1–33. https://doi.org/10.1371/journal.pgen.1006011

Suter, M., Ma, J., Harris, A., Patterson, L., Brown, K. A., Shope, C., Showalter, L., Abramovici, A., & Aagaard-Tillery, K. M. (2011). Maternal tobacco use modestly alters correlated epigenome-wide placental DNA methylation and gene expression. *Epigenetics*, *6*(11), 1284–1294. https://doi.org/10.4161/epi.6.11.17819

Svanes, C., Omenaas, E., Jarvis, D., Chinn, S., Gulsvik, A., & Burney, P. (2004). Parental smoking in childhood and adult obstructive lung disease: Results from the European Community Respiratory Health Survey. In *Thorax* (Vol. 59, Issue 4, pp. 295–302). https://doi.org/10.1136/thx.2003.009746

Svanes, C., Sunyer, J., Plana, E., Dharmage, S., Heinrich, J., Jarvis, D., De Marco, R., Norbäck, D., Raherison, C., Villani, S., Wjst, M., Svanes, K., & Antó, J. M. (2010). Early life origins of chronic obstructive pulmonary disease. *Thorax*, *65*(1), 14–20. https://doi.org/10.1136/thx.2008.112136

Tagiyeva, N., Devereux, G., Fielding, S., Turner, S., & Douglas, G. (2016). Outcomes of childhood asthma and wheezy bronchitis: A 50-year cohort study. *American Journal of Respiratory and Critical Care Medicine*, *193*(1), 23–30. https://doi.org/10.1164/rccm.201505-0870OC

Tai, A., Tran, H., Roberts, M., Clarke, N., Wilson, J., & Robertson, C. F. (2014). The association between childhood asthma and adult chronic obstructive pulmonary disease. *Thorax*, *69*(9), 805. https://doi.org/10.1136/thoraxjnl-2013-204815

Thyagarajan, B., Wojczynski, M., Minster, R. L., Sanders, J., Barral, S., Christiansen, L., Barr, R. G., & Newman, A. (2014). Genetic variants associated with lung function: The long life family study. *Respiratory Research*, *15*(134), 1–9. https://doi.org/10.1186/s12931-014-0134-x

Tomaki, M., Sugiura, H., Koarai, A., Komaki, Y., Akita, T., Matsumoto, T., Nakanishi, A., Ogawa, H., Hattori, T., & Ichinose, M. (2007). Decreased expression of antioxidant enzymes and increased expression of chemokines in COPD lung. *Pulmonary Pharmacology and Therapeutics*, *20*(5), 596–605. https://doi.org/10.1016/j.pupt.2006.06.006

Través, P. G., Luque, A., & Hortelano, S. (2012). Macrophages, inflammation, and tumor suppressors: ARF, a new player in the game. *Mediators of Inflammation*, *2012*. https://doi.org/10.1155/2012/568783

Upton, M. N., Smith, G. D., McConnachie, A., Hart, C. L., & Watt, G. C. M. (2004). Maternal and Personal Cigarette Smoking Synergize to Increase Airflow Limitation in Adults. *American Journal of Respiratory and Critical Care Medicine*, *169*(4), 479–487. https://doi.org/10.1164/rccm.200211-1357oc

Veldhuizen, E. J. A., & Haagsman, H. P. (n.d.). *Role of pulmonary surfactant components in surface ¢lm formation and dynamics*. www.elsevier.com/locate/bba

Vestbo, R., Prescott, E., Lange, P., Schnohr, P., Appleyard, M., Nyboe, rgen, & Gr, M. (1996). Association of Chronic Mucus Hypersecretion with FEV 1 Decline and Chronic Obstrurtive Pulmonary Disease Morbidity. *J Respir Crit Care Med*, *153*, 1530–1535.

Vibhuti, A., Arif, E., Deepak, D., Singh, B., & Qadar Pasha, M. A. (2007). Genetic polymorphisms of GSTP1 and mEPHX correlate with oxidative stress markers and lung function in COPD. *Biochemical and Biophysical Research Communications*, *359*(1), 136–142. https://doi.org/10.1016/j.bbrc.2007.05.076

Vucetic, Z., Carlin, J. L., Totoki, K., & Reyes, T. M. (2012). Epigenetic dysregulation of the dopamine system in diet-induced obesity. *Journal of Neurochemistry*, *120*(6), 891–898. https://doi.org/10.1111/j.1471-4159.2012.07649.x

Vucic, E. A., Chari, R., Thu, K. L., Wilson, I. M., Cotton, A. M., Kennett, J. Y., Zhang, M., Lonergan, K. M., Steiling, K., Brown, C. J., McWilliams, A., Ohtani, K., Lenburg, M. E., Sin, D. D., Spira, A., MacAulay, C. E., Lam, S., & Lam, W. L. (2014). DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. *American Journal of Respiratory Cell and Molecular Biology*, *50*(5), 912–922. https://doi.org/10.1165/rcmb.2013-0304OC

Walter, R. E., Beiser, A., Givelber, R. J., O'Connor, G. T., & Gottlieb, D. J. (2003). Association between glycemic state and lung function: The Framingham Heart Study. *American Journal of Respiratory and Critical Care Medicine*, *167*(6), 911–916. https://doi.org/10.1164/rccm.2203022

Wang, C. Di, Chen, N., Huang, L., Wang, J. R., Chen, Z. Y., Jiang, Y. M., He, Y. Z., & Ji, Y. L. (2015). Impact of CYP1A1 Polymorphisms on Susceptibility to Chronic Obstructive Pulmonary Disease: A Meta-Analysis. In *BioMed Research International* (Vol. 2015). Hindawi Limited. https://doi.org/10.1155/2015/942958

Wang, Y., Jia, M., Yan, X., Cao, L., Barnes, P. J., Adcock, I. M., Huang, M., & Yao, X. (2017). Increased neutrophil gelatinase-associated lipocalin (NGAL) promotes airway remodelling in chronic obstructive pulmonary disease. *Clinical Science*, *131*(11), 1147–1159. https://doi.org/10.1042/CS20170096

Wang, Y., Xu, J., Meng, Y., Adcock, I. M., & Yao, X. (2018). Role of inflammatory cells in airway remodeling in COPD. In *International Journal of COPD* (Vol. 13, pp. 3341–3348). Dove Medical Press Ltd. https://doi.org/10.2147/COPD.S176122

Wang, Z., Li, W., Guo, Q., Wang, Y., Ma, L., & Zhang, X. (2018). Insulin-Like Growth Factor-1 Signaling in Lung Development and Inflammatory Lung Diseases. In *BioMed Research International* (Vol. 2018). Hindawi Limited. https://doi.org/10.1155/2018/6057589

Warburton, D. (1983). Chronic Hyperglycemia with Secondary Hyperinsulinemia Inhibits the Maturational Response of Fetal Lamb Lungs to Cortisol. *J. Clin. Invest.*, *72*, 433–440.

Warburton, D., El-Hashash, A., Carraro, G., Tiozzo, C., Sala, F., Rogers, O., Langhe, S. De, Kemp, P. J., Riccardi, D., Torday, J., Bellusci, S., Shi, W., Lubkin, S. R., & Jesudason, E. (2010). Lung Organogenesis. *Current Topics in Developmental Biology*, *90*(C), 73–158. https://doi.org/10.1016/S0070-2153(10)90003-3

West, N. A., Crume, T. L., Maligie, M. A., & Dabelea, D. (2011). Cardiovascular risk factors in children exposed to maternal diabetes in utero. *Diabetologia*, *54*(3), 504–507. https://doi.org/10.1007/s00125-010-2008-1

Wiklund, P., Karhunen, V., Richmond, R. C., Parmar, P., Rodriguez, A., De Silva, M., Wielscher, M., Rezwan, F. I., Richardson, T. G., Veijola, J., Herzig, K. H., Holloway, J. W., Relton, C. L., Sebert, S., & Järvelin, M. R. (2019). DNA methylation links prenatal smoking exposure to later life health outcomes in offspring. *Clinical Epigenetics*, *11*(1). https://doi.org/10.1186/s13148-019-0683-4

Wongtrakool, C., Wang, N., Hyde, D. M., Roman, J., & Spindel, E. R. (2012). Prenatal nicotine exposure alters lung function and airway geometry through α7 nicotinic receptors. *American Journal of Respiratory Cell and Molecular Biology*, *46*(5), 695–702. https://doi.org/10.1165/rcmb.2011-0028OC

Yamada, L., & Chong, S. (2017). Epigenetic studies in Developmental Origins of Health and Disease: Pitfalls and key considerations for study design and interpretation. *Journal of Developmental Origins of Health and Disease*, *8*(1), 30–43. https://doi.org/10.1017/S2040174416000507

Yang, M., Cao, Z., Li, W., Zhou, J., Liu, J., Zhong, Y., Zhou, Y., Sun, L., Li, R., Cai, X., Xiao, H., & Zhou, A. (2024). Maternal Glycemia During Pregnancy and Child Lung Function: A Prospective Cohort Study. *Diabetes Care*, *47*(11), 1941–1948. https://doi.org/10.2337/dc24-0865

Yang, I. V., & Schwartz, D. A. (2011). Epigenetic control of gene expression in the lung. In *American Journal of Respiratory and Critical Care Medicine* (Vol. 183, Issue 10, pp. 1295–1301). https://doi.org/10.1164/rccm.201010-1579PP

Yoshikawa, M., & Yamakido, M. (2000). Microsomal epoxide hydrolase genotypes and chronic obstructive pulmonary disease in Japanese. In *INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE* (Vol. 5).

Zhang, Y. P., Liu, X. H., Gao, S. H., Wang, J. M., Gu, Y. S., Zhang, J. Y., Zhou, X., & Li, Q. X. (2012). Risk Factors for Preterm Birth in Five Maternal and Child Health Hospitals in Beijing. *PLoS ONE*, *7*(12), 1–7. https://doi.org/10.1371/journal.pone.0052780

Zhou, S., Wright, J. L., Liu, J., Sin, D. D., & Churg, A. (2013). Aging does not Enhance Experimental Cigarette Smoke-Induced COPD in the Mouse. *PLoS ONE*, *8*(8). https://doi.org/10.1371/journal.pone.0071410

Zou, Y., Chen, X., Liu, J., bo Zhou, D., Kuang, X., Xiao, J., Yu, Q., Lu, X., Li, W., Xie, B., & Chen, Q. (2017). Serum IL-1β and IL-17 levels in patients with COPD: Associations with clinical parameters. *International Journal of COPD*, *12*, 1247–1254. https://doi.org/10.2147/COPD.S131877