Investigating the Causal Impact of Gestational Diabetes on Youth-Onset Hypertension in Offspring

By

Nicole Brunton

A Thesis submitted to the Faculty of Graduate Studies of

the University of Manitoba

in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Applied Health Sciences

University of Manitoba

Winnipeg

Thesis Abstract

Hypertension is the second most common pediatric chronic disease worldwide. Previous work suggests that an individual's risk for hypertension could begin in utero. Early seminal studies demonstrated a link between exposure to malnutrition in utero and elevated risk for cardiometabolic abnormalities in the offspring. In the 21st century, studies revealed that hyperglycemia, pre-pregnancy diabetes, and gestational diabetes were all associated with markers of offspring cardiovascular health including increased blood pressure. However, it was not clear whether these associations represented a causal effect. Additionally, it is unclear the extent to which trends in gestational diabetes incidence have changed over time. This thesis was designed to fill these knowledge gaps. First, I conducted a descriptive study of gestational diabetes incidence to assess trends over time and among high-risk groups. Second, to investigate the causal impact of gestational diabetes on youth-onset hypertension among offspring, I conducted a triangulation study. Triangulation in epidemiology refers to conducting multiple studies, all designed to assess the same research question, that have differing limitations, risk of bias, and assumptions. For this thesis I conducted (1) an exact-matched study, (2) a negative exposure control study, and (3) a discordant sibling-matched study. Results showed that diagnosis of gestational diabetes has increased markedly over the last several decades with high-risk groups experiencing the highest rates and lower risk groups experiencing the highest increase in rates. Additionally, the evidence suggests that it is unlikely that exposure to gestational diabetes confers a direct independent increased risk for the development of youth-onset hypertension. Collectively, the work completed for this thesis demonstrates the importance of exploring causal factors that may simultaneously contribute to the cause of both elevated gestational diabetes and elevated youth-onset hypertension.

Acknowledgements

This thesis would not have been possible without the knowledge, expertise, and hard work of the incredible team at the Manitoba Centre for Health Policy. I would especially like to thank Mrs. Heather Prior and Mr. Kevin Friesen who were always gracious enough to provide thoughtful and thorough responses to my incessant questions. I would also like to extend a heartfelt thank you to all of the folks at DREAM and CHRIM. It has been an inspiring and motivating experience to be surrounded by so many brilliant, supportive, and collaborative individuals.

I am also grateful for all of the support I have received from every member of the McGavock lab during my tenure here. It is difficult to put into words exactly how much I value every coffee run and insightful conversation. Suffice it to say, they were crucial to my success in this program.

To my PhD committee – Dr. Nathan Nickel, Dr. Allison Dart, and Dr. Todd Duhamel – I cannot thank you enough for the support and guidance you provided me throughout my program. I am a better researcher because of the thoughtful comments, questions, and advice you have been generous enough to share with me. I would like to especially thank Dr. Jon McGavock. Your unwavering support was paramount to my completing this thesis, and I am deeply grateful for your mentorship.

To my family and friends, new and old, I cannot imagine a world in which I would have been able to complete this degree without you. Thank you.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1 Thesis overview

There is a consensus among some researchers that complex chronic diseases like hypertension may originate during gestation. Early studies of famine repeatedly demonstrated significant relationships between a disturbed intrauterine environment and poorer offspring health outcomes,[1-4] including elevated blood pressure.[5-7] In the 21st century however, maternal overnutrition and accompanying metabolic abnormalities like maternal diabetes have become the dominant intrauterine concerns for long term chronic disease risk among offspring.[8] There is a dense body of epidemiological[9-12] and mechanistic evidence[13-17] that describe early life origins of hypertension secondary to diabetes exposure in utero; however, it remains unclear whether these data represent a cause and effect relationship.

In observational research, sometimes correlation does imply causation. However, causality should only be assumed under certain circumstances, which are still largely debated.[18] Most evidence surrounding in utero exposure to diabetes and subsequent risk for hypertension in humans is correlational; yet these data have been discussed extensively using causal language without clearly describing the nuanced circumstances or assumptions underlying the causal interpretations.[19] Unfortunately, there are methodological challenges associated with studying in-utero exposures and offspring health outcomes that make causal inference difficult. For example, randomized controlled experiments are typically unethical, impractical, or prohibitively expensive. One way to overcome this limitation is to triangulate evidence from multiple observational study designs that each have different limitations, biases, and assumptions to give

a more robust understanding of the validity of the causal relationships of early life exposures and chronic disease outcomes.[20]

The present thesis project represents an intersection of the life course approach to studying chronic disease and causal inference in epidemiology. In this context, my aim was to investigate the validity of the causal association between exposure to diabetes in utero and risk of hypertension among offspring. I also present a description of gestational diabetes over the last several decades in the population of Manitoba to provide context for the primary exposure of interest.

1.2 Organization of dissertation

This dissertation is a grouped manuscript, or "sandwich style", thesis based on the description by the University of Manitoba Faculty of Graduate Studies. The dissertation contains nine chapters. The first three chapters are: (1) the introduction, (2) the literature review, and (3) the hypothesis and specific objectives, followed by two manuscripts (chapters 4 and 5). Chapter 6 consists of an extended discussion and concomitant interpretation of study results followed by a concluding chapter which summarizes key findings, limitations, and future directions (Chapter 7). Chapter 8 and 9 contain all of the references and all of the supplementary material for each of the previous chapters, respectively. The manuscript chapters contain a preface explaining the contributions of myself and the co-authors and a publication status statement (i.e., not-submitted, in-review, etc.).

My primary goal with this dissertation was to take a triangulation approach to hypothesis testing using population health data. Therefore, the manuscript in Chapter 5 contains information on three distinct studies and is considerably longer than the other chapters. The reason for

combining these studies into one chapter rather than three separate chapters is because I intend to publish this work as one manuscript to ensure the relationship between the studies and triangulation is clear to readers. The initial study (Chapter 4) provides a descriptive analysis of the trends in the exposure variable in Manitoba over the last few decades to provide context for the causal investigation. Quantitatively, the studies in Chapter 5 are triangulated simply by comparing the primary results from each study to assess how many, out of the three, support or refute the hypothesis with some discussion of the sensitivity analyses, strengths, and limitations of each study. Chapter 6 is an extended discussion of the findings from Chapter 4 and 5, with additional consideration of the various sensitivity analyses and sources of bias for each study methodology.

Chapter 2: Literature Review

2.1 Chapter overview

The purpose of this literature review is to provide relevant background information on the theories and previous studies that informed the present work. The first two sections review the theoretical background. Primarily, the focus will be on the Developmental Origins of Health and Disease framework and life course epidemiology, followed by a brief overview of the developments in casual inference related to early life origins of disease and this project. The final sections provide a review of the literature surrounding the primary exposure and outcome of interest – gestational diabetes and youth-onset hypertension among offspring, respectively – describing current understanding, gaps, and limitations. Finally, a chapter summary at the end reiterates key points from the review.

2.2 Developmental origins of health and disease

Early studies of famine repeatedly demonstrated significant relationships between a disturbed intrauterine environment and poorer health outcomes for the offspring,[1-4] including elevated blood pressure.[5-7] However, in the 1980s, Dr. David Barker was one of the first to popularize the idea that an individual's risk for several chronic diseases could begin early in life, after being exposed to an adverse intrauterine environment.[21] This led to the establishment of the International Council on the Fetal Origins of Adult Disease in 1999, which was later expanded to Developmental Origins of Health and Disease upon recognizing that there are other critical periods of development where environmental exposures could produce long-term effects, both positive and negative.

Developmental origins research typically investigates how the prenatal and early postnatal environments influence health, wellbeing, and later risk of noncommunicable diseases. The International Developmental Origins of Health and Disease Society postulates that environmental adversity during critical developmental windows can impact growth trajectories and "program" adverse health outcomes (Figure 2.1).[22, 23] The developmental origins community is largely concerned with elucidating the physiological basis by which these early life exposures influence development and contribute to chronic disease risk. Since this approach to studying chronic disease was initially adopted, several interrelated concepts and theories have been developed to study a wide array of exposures and diseases. Although the exact meaning of developmental origins of disease differs slightly among researchers, the concept, largely shaped by Dr. David Barker's early work, has remained the same.

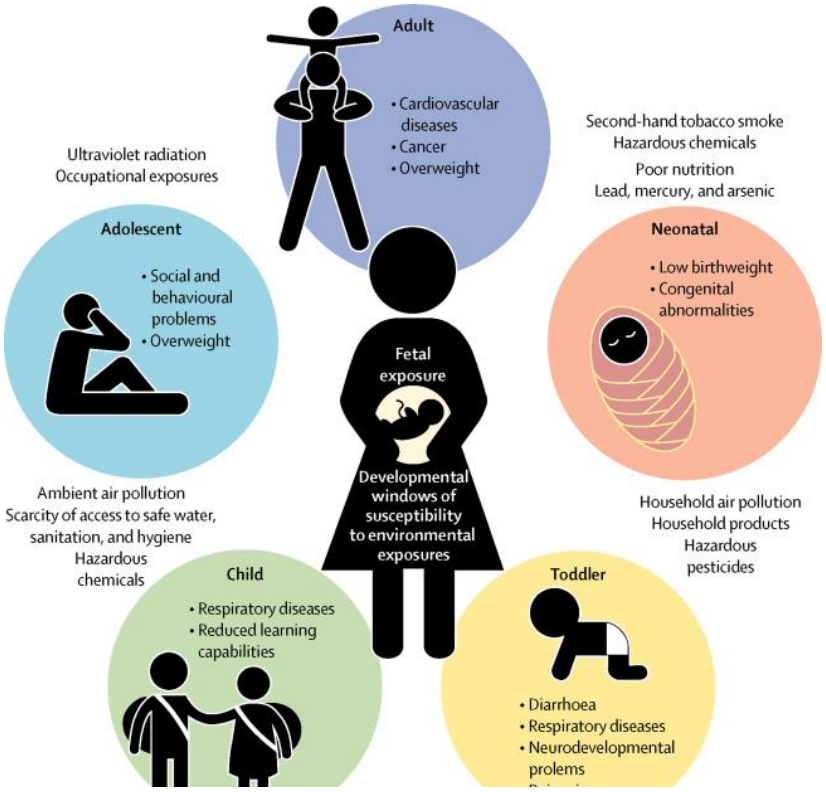

Figure 2.1 Early life environmental exposures can increase offspring's susceptibility to later environmental challenges that are immediate or accumulate over time to increase disease risk. Figure reproduced from Poore et al. (2017).[23]

2.2.1 Fetal origins of adult disease

Developmental Origins of Health and Disease, as it is known today, began as the Fetal Origins of Adult Disease, which is often colloquially referred to as "The Barker Hypothesis". Dr. David Barker was a physician and epidemiologist who popularized the notion that important factors during fetal life, particularly nutrition, could "program" adult cardiovascular disease.[24] Barker published a series of seminal papers in the late 1980s and early 90s that are still routinely referenced over 35 years later.

Prior to Barker's work, in 1962, geneticist Dr. James Neel proposed the "thrifty genotype" hypothesis to explain the modern chronic disease epidemic, particularly increasing rates of diabetes.[25] The "thrifty gene" refers to having a genetic predisposition for storing energy as fat, which was advantageous for early hunter-gatherers when meals were sparse. In contemporary times where food is easily accessible and starvation is rare, the "thrifty gene" became a source of increased disease risk.[25-27] Dr. Neel later reconsidered his hypothesis[28] and the "thrifty phenotype" hypothesis by Drs. Barker and Hales replaced it.[29]

Dr. Barker and Dr. Hales argued that a genetic component could not explain the rapid change in disease incidence. Instead, they proposed that exposure to an adverse intrauterine environment alters fetal development, which subsequently increases disease risk. The "thrifty phenotype" described adaptive developmental changes in response to adverse pre- and early postnatal environments, however these phenotypic changes are maladaptive when the later environment is adequate.[26, 29-31] By the late 90s the thrifty phenotype hypothesis began to expand from epidemiology into other areas of perinatal science and collectively become the Fetal Origins of Adult Disease. In 2003, the fetal origins hypothesis was reformed again and became

an academic society called The International Society for Developmental Origins of Health and Disease.[32] "Developmental origins" replaced "fetal origins" to include important extrauterine exposures (e.g. conception, infancy, and puberty) and "health" was added to acknowledge that early life exposures also have implications for health and well-being.[33]

2.2.2 Predictive and immediate adaptive responses

Dr. Peter Gluckman and colleagues proposed the predictive adaptive response hypothesis to further describe the mechanisms linking intrauterine exposures to future disease risk.[26] The predictive adaptive responses hypothesis extends the thrifty phenotype hypothesis to include any period of developmental plasticity, and the future environment does not necessarily refer to the early postnatal period.[32, 34] For example, the number of sweat glands is set in the first year of life by the ambient temperature. An individual born in a cold climate would develop fewer sweat glands and would be predisposed to heat stress if they later moved to a hot climate. When the early life adaptive response to the cold environment does not match the current or future hot environment it becomes maladaptive.[26] In other words, a fetus may adapt to a certain environment in anticipation that the future environment will be similar. However, if there is a mismatch, that individual might be at higher risk for developing disease.

In addition to predictive adaptive responses, there are also immediate adaptive responses, which refer to phenotypic responses, or "coping", to an acute environmental challenge. These responses promote immediate survival but may permanently alter development and be detrimental to health, regardless of the postnatal environment. For example, an immediate adaptive response in pregnancy would be fetal growth restriction in response to maternal starvation. The fetus' growth is slowed to conserve energy in a low nutrient environment and

promote survival, however this growth restriction that was adaptive at the time of the adverse exposure (e.g., altered kidney development and function[35, 36]) is maladaptive postnatally.[32] Several long-term follow-up studies of offspring exposed to starvation in utero during World War II further demonstrate the negative health impacts following development in a low-nutrient environment.[6, 37] Predictive and immediate adaptive responses are not always mutually exclusive. Often, there is considerable overlap in predictive and immediate adaptive responses that may collectively help explain many of the observed associations between intrauterine exposures and offspring health outcomes.

In the 21st century, maternal overnutrition and accompanying metabolic abnormalities like maternal diabetes have become the dominant intrauterine exposures believed to influence long term chronic disease risk among offspring.[8] One of the main criticisms of predictive adaptive responses is that it cannot fully explain the increased risk for cardiovascular disease among offspring exposed to overnutrition pre- and postnatally.[13] For example, offspring exposed to overnutrition in utero should, according to the predictive adaptive response theory, do better when exposed to overnutrition postnatally compared to offspring not exposed to overnutrition in utero. However, it appears that the opposite is true; offspring exposed to adequate nutrition in utero do better almost universally.[38] It may be the case that intrauterine immediate and predictive adaptive responses play a role in certain exposures and outcomes. However, other potentially harmful environmental exposures during critical developmental windows may prevent offspring from achieving their full mental and physical potential via their epigenome.[39]

2.2.3 Critical windows theory

The critical windows theory extends the immediate and predictive adaptive responses theories by providing a mechanism – alterations to the epigenome – that may be driving the adaptive responses to the altered intrauterine environment. The epigenome is a collection of chemicals and proteins that can act on the genome and cause stable alterations in gene expression without concomitant changes to the nucleotide sequence.[40] Epigenetic regulation occurs during "critical windows" of cellular proliferation and differentiation,[41, 42] and this developmental plasticity allows for several phenotypes to result from a single genotype.[34, 43] These critical windows of development include the periconceptional period, gestation, the early postnatal period, and puberty.[44, 45] Depending on the timing and exposure or environmental cue, an

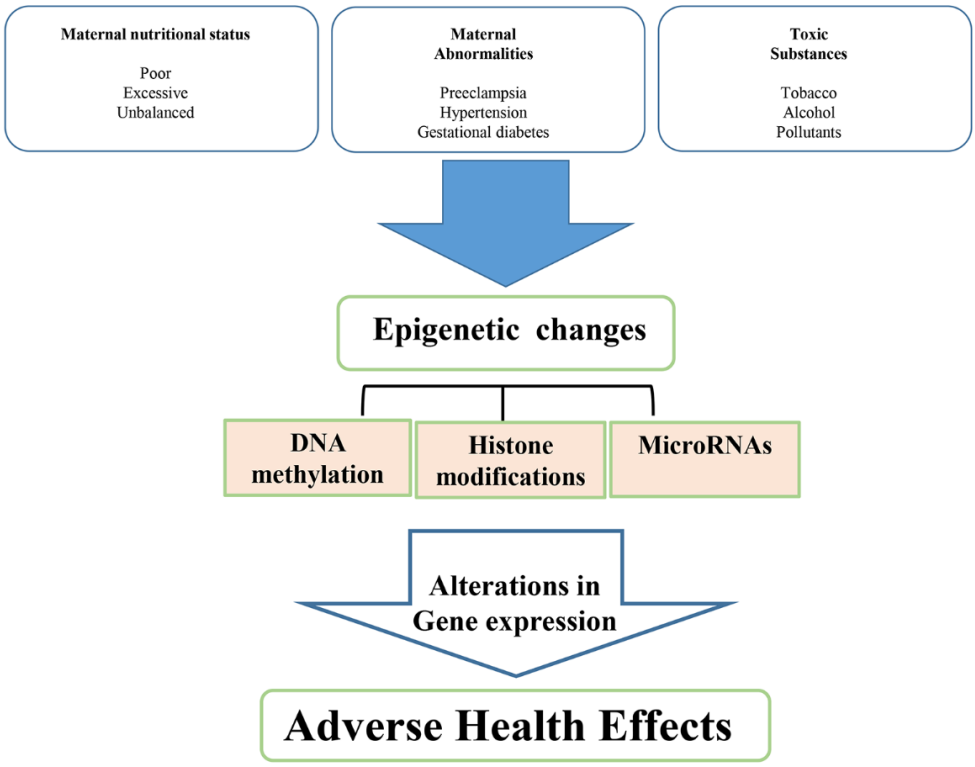

Figure 2.2 Adverse environmental exposures leading to changes in the epigenome resulting in adverse health effects later in life. Figure reproduced from Deodati et al. (2019).[41]

individual can develop a functionality that predisposes them to developing chronic disease (Figure 2.2).[41]

Once epigenomic alterations to the genome occur the genome is considered to be "marked" and these epigenetic marks can be transmitted through the next generation.[46] Several studies examining individuals who were starved during the Dutch Hunger Winter in 1944-45 have reported epigenetic changes in the adult offspring who were exposed to famine during pregnancy[47, 48] and several of these epigenetic changes were found to mediate associations with growth and metabolism of those exposed offspring.[49] Additionally, studies have demonstrated an association between exposure to gestational diabetes and altered epigenetic markers in placental or cord blood of those offspring.[50-53] There is also evidence of epigenetic changes in adolescents and adults following exposure to gestational diabetes.[54] However, the specific markers are not typically consistent across life stages (i.e., from fetal cord blood to adulthood),[54] suggesting that other environmental cues during critical developmental windows could further alter epigenetic marks and modify chronic disease risk.

The critical windows theory relies on the hypothesis that epigenetic marks are the main mechanism through which environmental exposures have lasting impacts on future disease risk. However, given the relative novelty of examining the epigenome in a developmental origins of disease framework, it remains unclear how deterministic these epigenetic marks are over the life course of an individual in response to an intrauterine exposure.

2.2.4 Life course epidemiology

One of the primary limitations of the predictive and adaptive responses and critical windows theories is their deterministic nature and lack of consideration for time-varying

exposures throughout life. Life course epidemiology takes a broader approach by considering long-term biological, behavioural, and psychosocial processes that occur in response to exposures throughout the life span from preconception to adult life.[55] Life course epidemiology was developed in parallel with the Fetal Origins of Adult Disease hypothesis (i.e., the Barker hypothesis), which subsequently became Developmental Origins of Health and Disease. However, since its inception, life course epidemiology incorporated both social and biological pathways with additional attention paid to later life interventions to alter disease risk or progression, whereas developmental origins hypotheses tend to remain focused on early life "programming" and is somewhat dismissive of non-biological pathways.[56]

Technically speaking, the field life course epidemiology encompasses the previously discussed theories but places them within a broader context. The term was originally coined to reduce the polarization that was occurring between epidemiologists who supported early-life programming of chronic disease and those who endorsed adult-life hypotheses.[55] Thus, several life course models have been developed to conceptualize the natural history of various chronic diseases. A detailed review of the various life course models can be found elsewhere;[55] however, they are generally categorized as either belonging to a critical period or accumulation of risk pathway.

The critical period pathways are inclusive of the adaptive responses and critical windows theories discussed above. These models suggest that there are critical and sensitive periods whereby individuals are particularly vulnerable to developing adverse functionality in response to certain environmental exposures and the result of this exposure is fixed. There are also models within this pathway that describe how effect modification can occur later, following an exposure inscribed during fetal development. In these models, the modifier can either amplify or

counteract the underlying dysfunction or developmental abnormality. The accumulation of risk models posits that multiple risk factors, both biological and social, contribute to the development of disease under varying degrees of connectedness. The multiple exposures could be unrelated, correlated, additive, or mediating (where exposure to variable one leads to exposure to variable two, and so on). A graphical depiction of the accumulation of risk models can be found below in Figure 2.3.[55]

A considerable strength of working within the life course epidemiology approach to studying in-utero exposures and long-term health outcomes in the offspring is the comprehensive nature of the field. Considering the complex network of biomedical, psychosocial, and behavioural factors that contribute to health is crucial to understanding the natural history of several chronic diseases, particularly noncommunicable disease like cardiovascular disease.

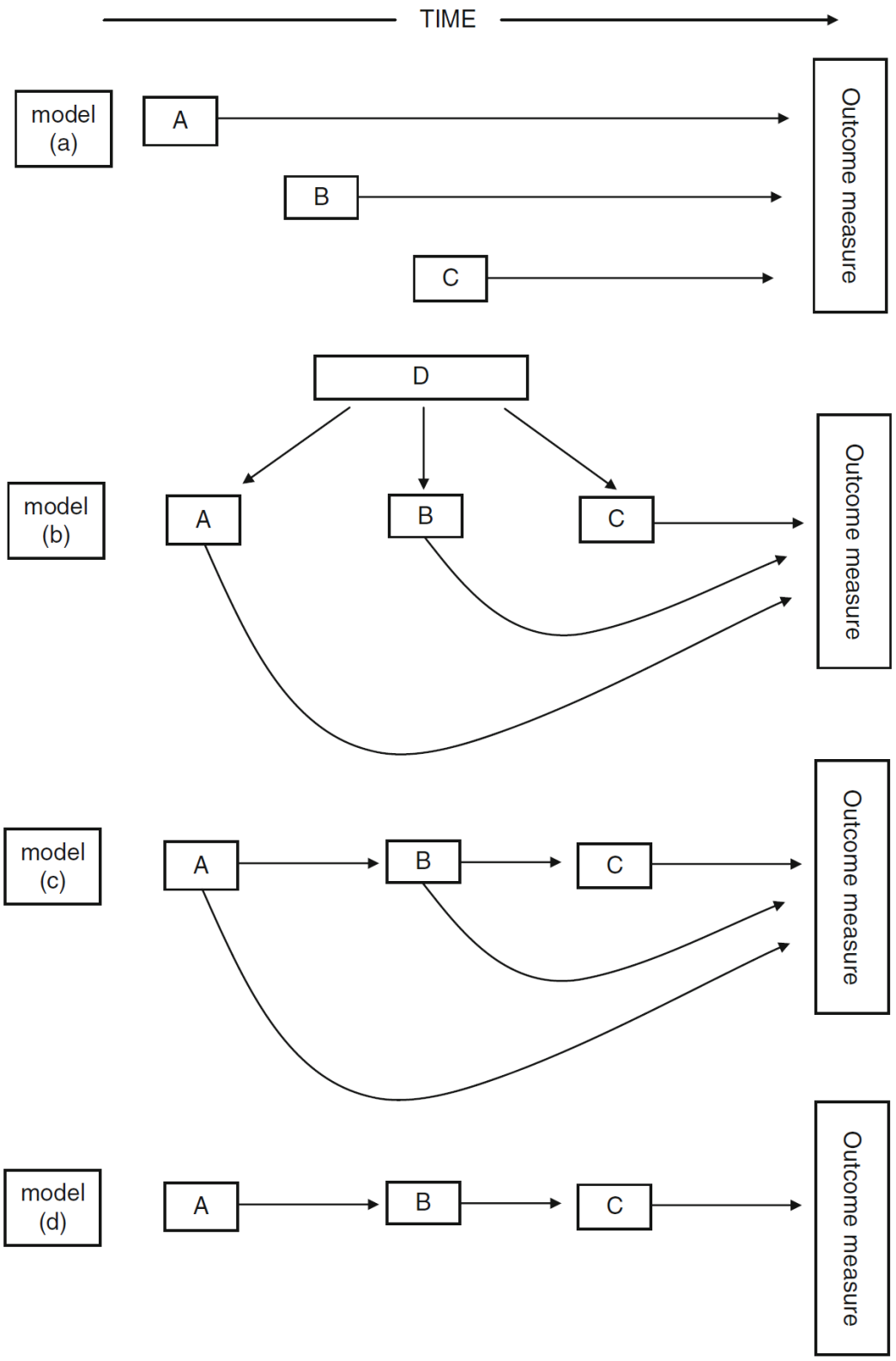

Figure 2.3 A graphical representation of the accumulation of risk models. (a) accumulation of risk with uncorrelated exposures, (b) accumulation of risk with correlated exposures, (c) chain of risk additive model, and (d) chain of risk model. Figure reproduced from Ben-Shlomo et al. (2023).[55]

2.3 The importance of understanding the causes of pediatric hypertension

The prevalence of hypertension among children and adolescents has increased over the last several decades[57] with specific estimates ranging from 2-6% in the general pediatric population.[58-61] A diagnosis of hypertension during adolescence is particularly concerning because adolescents with hypertension or pre-hypertension display early signs of vascular disease including increased carotid intima media thickness.[62] Additionally, a recent long-term follow up cohort study demonstrated that adolescents with hypertension are at greater risk of stroke, myocardial infarction, and congestive heart failure, compared to normotensive adolescents.[63] Based on the acute and long-term consequences of hypertension in adolescence, understanding early life modifiable causes could inform prevention strategies.

In 2017, the American Academy of Pediatrics published new guidelines for the diagnosis of pediatric hypertension in which children with overweight or obesity were not included in the reference data.[64] This change shifted the percentile curves downward so that children who were reclassified as having elevated blood pressure or hypertension represented a high risk group including those with higher body mass index.[58] Additionally, the updated criteria for diagnosing hypertension in youth aged 13 years or older aligns with adult cut-offs, simplifying diagnosis for clinicians.[65] However, screening for elevated blood pressure in youth remains low. The annual mean rate of blood pressure measurement remained around 30% in Canada between 2011-2017, and nearly half of children with overweight did not have a measurement recorded.[65]

Early screening for elevated blood pressure in children is vital because it enables earlier detection and intervention. Otherwise, the consequences of having elevated blood pressure as a child or adolescent can be severe. Hypertension among children can lead to short term risks of

target organ damage and long-term risks of developing cardiovascular disease as adults[66] and premature death due to myocardial infraction, heart failure, stroke, or end-stage kidney disease.[67] There are several risk factors that cluster around youth with hypertension and high blood pressure; however, the specific etiology is complex and multifactorial.

Pediatric hypertension, particularly primary hypertension, tracks closely with the obesity epidemic and is more common among youth with poor dietary behaviours (e.g., high salt intake, high consumption of sweetened beverages, and lower fruit and vegetable intake),[68] lower physical activity levels, and poor sleep habits.[69] A large proportion of pediatric hypertension cases, especially among young children, are also secondary to other conditions, namely renovascular disease.[70] Importantly, several of the risk factors and primary causes of pediatric hypertension noted above are associated with intrauterine exposures[71-74]. Moreover, there are several observational data suggesting a disturbed fetal environment may be directly linked to increased susceptibility to high blood pressure.[11, 12, 75, 76] In order to improve our understanding of the etiology of pediatric hypertension it is important elucidate the role of potential upstream causes like intrauterine exposures to enhance intervention and prevention strategies.

2.4 Exposure to diabetes in utero as an upstream cause of pediatric hypertension

Several researchers have taken a life course approach to studying non-communicable diseases to help explain the recurring associations observed between various in-utero exposures and offspring health outcomes.[33, 77] Cardiovascular disease, and especially blood pressure, was an early topic of study among developmental origins researchers[5, 7, 78] and continues to occupy the field today. More recently there has been a greater focus on childhood and adolescent blood pressure as hypertension is one of the most common pediatric chronic diseases worldwide.[58]

Early observational studies,[7, 79] natural experiments,[3, 6, 37] and animal models[80, 81] have demonstrated that offspring exposed to famine in utero were more likely to develop cardiovascular risk factors, like hypertension, as adolescents and in middle age. Recent investigations into the other end of the diet exposure spectrum, that is overnutrition, found similar associative evidence between maternal weight or body mass index and offspring risk of elevated blood pressure.[82, 83] A closer look into the metabolic abnormalities that increasingly complicate pregnancies,[84] with or without concomitant obesity, revealed that hyperglycemia,[85] hyperinsulinemia,[74] pre-pregnancy diabetes,[12] and gestational diabetes[11] are all associated with various markers of offspring cardiovascular health including increased blood pressure, a more atherogenic lipid profile, and cardiac or vascular dysfunction.

There is considerable epidemiological evidence of a relationship between exposure to a disturbed metabolic milieu in utero and offspring blood pressure. Many epidemiological studies have assessed this exposure as a binary disease state assessing type 1 diabetes, type 2 diabetes, or gestational diabetes and have demonstrated an elevated risk of blood pressure and hypertension among exposed offspring.[11, 12, 71] Others have studied specific facets of diabetes exposure including hyperinsulinemia and found similarly elevated risk of cardiovascular disease markers in offspring.[74] However, hyperglycemia is widely cited and likely the most common metabolic disturbance among diabetic pregnancies that is associated with increased risk of elevated blood pressure in offspring.[76]

Many mechanisms have been proposed to explain the relationship between maternal metabolic abnormalities and increased cardiovascular disease risk among offspring. The primary mechanisms reported in the literature include altered organ development[86] especially reduced nephron number,[87] increased angiotensin converting enzyme activity,[76] and increased vascular

reactivity to vasoconstrictors.[86] Animal models have shown that rat offspring of high-fat fed dams with diabetes had adverse cardiac structure[88] and altered function when exposed to high-fat diets.[13, 89] Similarly, vascular remodelling including increased intima media thickness, vascular stiffness, and decreased endothelial cell number has been observed across species following exposure to a high-fat diet in utero.[86, 90] Some human studies have also reported altered endothelial tube formation among offspring exposed to gestational diabetes[17] and increased arterial stiffness following in utero hyperinsulinemia exposure.[74] Another study reported similar cardiac and vascular function among children exposed to diabetes in utero compared to control, however exposure to poorly controlled diabetes was associated with worse cardiovascular outcomes.[91] The mechanisms described in the literature to date generally fall under immediate adaptive responses, (similar to critical period pathways in life course epidemiology), in that they represent permanent fetal maladaptation to the disturbed intrauterine environment.

Evidence for the mechanisms described above comes largely from animal studies, and evidence in humans is lacking or considerably more varied. For example, some studies reported no difference in cardiac function in children exposed to diabetes in utero compared to controls.[92, 93] Also, altered renal structure, which has also been implicated as a mechanism for increasing cardiovascular disease risk factors among offspring, appears to be more highly correlated with premature birth rather than direct intrauterine programming.[94] Other studies suggest that renal structure and function may be altered in offspring via hormonal abnormalities in utero.[95] Additionally, there is a growing body of literature challenging the validity of direct causal relationships between many in utero exposures and offspring blood pressure.[83, 96-98]

Over the past decade the validity of seminal developmental origins research has been challenged.[99-101] More and more studies are providing conflicting evidence in support of a direct

intrauterine mechanism leading to altered offspring development. A recent study I led using a novel counterfactual-based causal mediation analysis, failed to show a direct effect between maternal pre-pregnancy body mass index and elevated offspring blood pressure in adolescence.[102] Increasingly, studies are providing conflicting evidence of developmental origin relationships including in utero diabetes exposure and offspring hypertension.[97, 98] Measurement error, misclassification of the exposure or outcome, and confounding bias likely contribute to these conflicted findings, therefore it remains unclear whether exposure to hyperglycemia in utero causally increases offspring risk for hypertension.

2.5 Causal inference and developmental origins

Investigations into the developmental origins of chronic disease are inherently causal as researchers attempt to elucidate the causal disease pathway. Unfortunately, there are specific methodological challenges associated with this field of study, particularly that randomized controlled trials are typically not feasible because they are unethical, impractical, prohibitively expensive, or various combinations of the three. Fortunately, around the same time that the fetal origins hypothesis and life course epidemiology fields were becoming established there was a concomitant emergence in causal models for epidemiology.[103-106] In addition to formally defined terminology and models (e.g., Rubin's potential outcomes model,[107, 108] Pearl's counterfactual model,[109-111] and causal diagrams like directed acyclic graphs[104, 112]), there has been a greater emphasis placed on evidential pluralism where multiple forms of evidence are utilized to draw causal conclusions.[18] However, studies of the early origins of youth-onset hypertension have been slow to adopt these causal frameworks.

Causal interpretations of several developmental origin of disease studies are clear from the use of causal language such as early adoption of the term “programming” to describe the relation between maternal variables and offspring health outcomes, however few, if any, studies explicitly discussed causality. There is no consensus on what constitutes “causation”, and no single theory or definition of “cause” is widely recognized. That being said, there are five general categories that can be used to succinctly summarize the different types of causal evidence: (1) regularity, (2) counterfactual, (3) probabilistic, (4) mechanist, and (5) intervention (Table 2.1).[18] Although these categories are not always mutually exclusive it is helpful to think about and discuss causal inference using specific terms. Many studies that have investigated intrauterine exposures and offspring health outcomes later in life have relied on probabilistic and mechanistic evidence to infer causation (though not discussed explicitly).

Table 2.1 Summary of causal evidence categories.

Evidence category	Description
1. Regularity	Causes are repeatedly and reliably followed by their effects while coincidental pairings do not typically reoccur.
2. Counterfactual	We can never observe an individual under more than on condition simultaneously so we must consider what *would have* happened (i.e., if the cause had not happened, the effect would not have happened).
3. Probabilistic	If A causes B, then we would observe that A typically raises or lowers the probability of B occurring.
4. Mechanistic	Identify a basis for causation through a physical property that is a mark of transmission between the cause and the effect.
5. Intervention	If we intervene on a particular cause, then we will observe a change in the effect (i.e., if we alter X, we will see a subsequent change in Y).

An important causal concept that is not often discussed in developmental origins research studies that is applied in the present thesis is counterfactuals. A counterfactual approach to causal inference is about determining what might have happened had the exposure status of the individual been different. The fundamental problem of causal inference, according to statistician Paul Holland, is that you can never observe an individual experience more than one condition simultaneously, and thus you can never determine individual-level causal effects.[113] Imagining and quantifying counterfactual scenarios is important for causal inference because we can estimate causal effects by comparing what we observed to what we might have observed in the counterfactual world. Since we can never observe an individual experience more than one scenario simultaneously,[113] we must always estimate the counterfactual. If an exposure is causal, then we would expect the outcome (the effect) to differ between the counterfactual and observed scenarios. The causal evidence is only as good as the counterfactual estimate which hinges on several important assumptions depending on the specific method being used.

Inferences made under the counterfactual causal framework relies on various assumptions, and most are specific to the study design or statistical method used. One assumption however, the stable unit treatment value assumption (SUTVA), applies to almost all models. SUTVA is satisfied when the exposure is assumed to be consistent regardless of the exposure assignment mechanism, and when the exposure status of one individual does not alter the exposure effect of another individual, in other words there is no interference.[114, 115] If the SUTVA assumption is satisfied, in addition to method-specific assumptions, then we can make reasonable causal inferences using observational data. Importantly, we are assessing population rather than individual-level causal effects, meaning that the cause or effect concerns a group of individuals (e.g., an increase in the rate of diabetes in pregnancy causes, on average, an increase in the prevalence of hypertension among those offspring). This is relevant because hyperglycemia in pregnancy is not a necessary or sufficient cause,[116] meaning that the outcome can still occur without the exposure and having the exposure alone does not guarantee the outcome will occur. The relevance of this relationship is whether exposure to hyperglycemia in pregnancy is a causal component of offspring hypertension and if, on a population level, changing rates of maternal diabetes in pregnancy will have an accompanying valid effect on offspring hypertension.

As alluded to above, a vital component to studying the natural history of complex chronic disease is incorporating multiple sources of evidence. Triangulation in epidemiology is a method that can improve our ability to infer causation from observational data. Triangulation in epidemiology refers to simultaneously interpreting evidence from multiple studies designed to assess the same hypothesis but have differing sources of bias, assumptions, and limitations.[117] Triangulation has been applied in etiological epidemiology to assess multiple intrauterine

exposures including smoking,[118] stress,[119] and body mass index.[120] To our knowledge, a similar approach has not been applied to the exposure of gestational diabetes and cardiovascular disease specific outcomes, including youth-onset hypertension.

2.6 Chapter summary

It is useful to think about intrauterine exposures and offspring outcomes in the context of the theories described above. Life course epidemiology, in particular, provides a comprehensive framework from which we can consider and describe how certain early life exposures may lead to chronic disease, whether directly or through interactions with other factors. There is a large body of literature that supports a relationship between exposure to gestational diabetes and offspring hypertension, though it is methodologically difficult to tease out valid causal relationships. Therefore, we must consider various types of causal evidence to study complex chronic disease like hypertension.

Chapter 3: Specific Objectives and Hypotheses

3.1 Theoretical framework

The theoretical framework underlying this project is influenced by both the critical period and accumulation of risk pathways described within life course epidemiology and the concept of evidential pluralism for causal inference. I posit that there is an abundance of evidence in the literature describing a critical time during gestation where exposure to diabetes can impair fetal development resulting in elevated risk for cardiovascular disease, namely elevated blood pressure and hypertension. However, we also acknowledge the limitations in these data and the contradictory findings that have been published more recently. Therefore, the plurality of evidence is key for making valid causal inferences.

The advantage of working within the life course epidemiology framework is that it highlights the importance of considering both the biomedical intrauterine mechanisms for chronic disease susceptibility and the extrauterine psychosocial and behavioural factors. Figure 3.1 provides a graphical overview of my theoretical framework demonstrating how exposure to gestational diabetes in utero could predispose an individual to developing hypertension. In the figure, two hypothetical individuals discordant for exposure to gestational diabetes in utero are exposed to identical environments that either increase or decrease their risk of developing hypertension. The individual exposed to gestational diabetes in utero has a higher baseline risk of developing hypertension and thus, has a greater relative risk throughout their life course compared to the unexposed individual (despite additional variables that interact to either increase or decrease hypertension risk further). Whether the initial baseline risk is in fact higher among offspring exposed to gestational diabetes is the primary focus of this thesis.

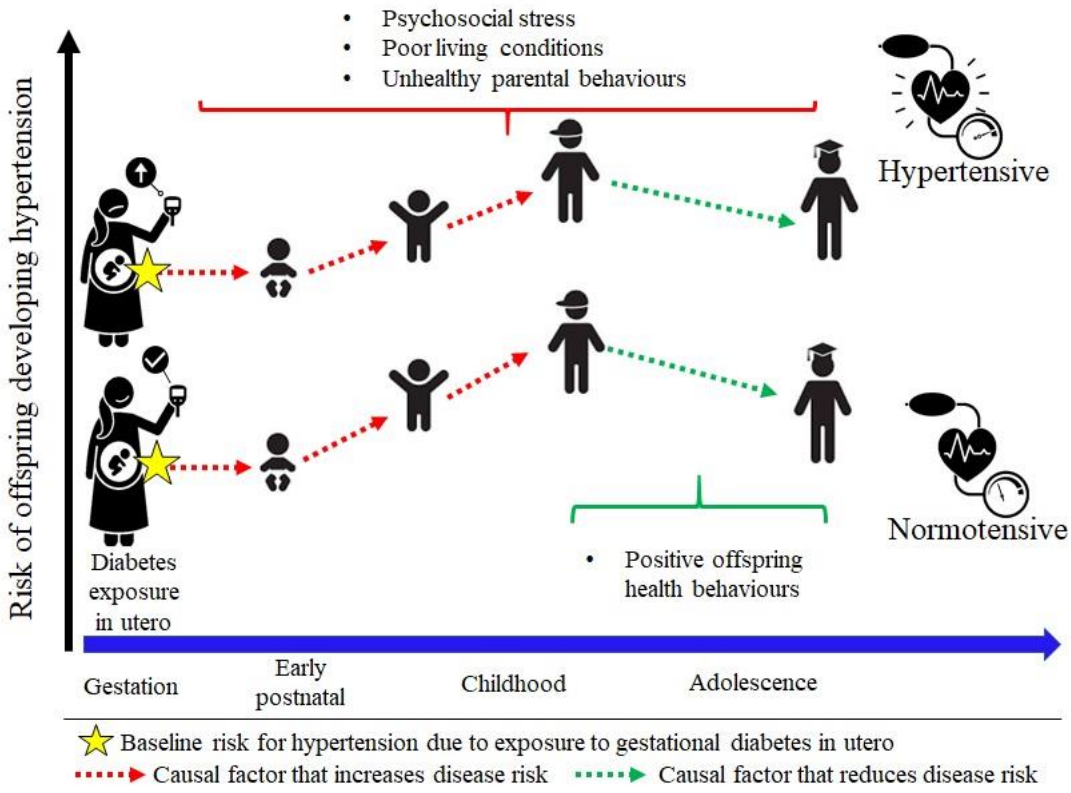

Figure 3.1 Theoretical framework

3.2 Hypothesis and study objectives

The primary objective of this project is to test the critical period theory that exposure to diabetes in utero increases offspring risk of developing youth-onset hypertension. The secondary objective of this thesis is to describe population trends in gestational diabetes incidence over the last several decades in order to provide context for the potential impact of the causal investigation. In keeping with the abundance of literature, my overarching hypothesis is that exposure to gestational diabetes during pregnancy will be causally associated with elevated rate of youth-onset hypertension in offspring.

Chapter 4: Trends in Gestational Diabetes Incidence in Manitoba from 1981 – 2019: A Descriptive Study

Authors: Nicole M. Brunton, MSc; Kevin J. Friesen, MSc; Jennifer M. Yamamoto, MD; Heather J. Prior, MSc; Jonathan McGavock, PhD.

Manuscript status: Under review

4.1 Contribution of authors

I conceived of this study with support from Dr. McGavock, Dr. Yamamoto, and Mrs. Heather J. Prior at MCHP who were working with similar data on a separate existing project. I lead the development and revisions of the objectives, included variables, and analysis plan. I was the primary liaison with the lead analyst, Mr. Kevin Friesen, and was responsible for all data visualization and interpretation related to this project. I created all the figures and tables, wrote the original draft, and coordinated the revisions. All authors contributed to editing the original draft of the manuscript and approved the final draft for submission to the Canadian Journal of Diabetes.

4.2 Preface to manuscript

As described in previous chapters, trends in gestational diabetes are increasing internationally,[121] nationally,[122] and across provinces.[123-125] However, to our knowledge, the most recent data on gestational diabetes incidence in Manitoba was published in 2008.[126] As this thesis is centered on elucidating the implications of exposure to gestational diabetes on offspring cardiovascular health later in life, it is important to understand the trends in gestational diabetes over the last several decades. This descriptive study provides the context of the primary exposure

that will be utilized in the subsequent thesis studies. Results from this study will contribute to the interpretation and broader understanding of all results hereafter.

4.3 Manuscript

Abstract

Background: Incidence of gestational diabetes is increasing globally. However, there is little information describing trends in gestational diabetes incidence within sub-groups that often experience health inequities.

Methods: We performed a serial descriptive study within a population-based administrative health database to describe trends in gestational diabetes incidence between 1981-2019, stratified by sub-populations based on age, urbanicity, and neighbourhood-level average household income. We calculated yearly incidence across sub-groups and annual percent change in incidence to assess trends over time. Geospatial mapping was used to visualize trends by geographical area.

Results: Annual gestational diabetes incidence increased among pregnancies from 1.32% (n=12,342) in 1981 to 8.65% (n=12,434) in 2019 with an inflection occurring around 2010. This trend was observed after stratifying by age, urbanicity, income, and socioeconomic status (SES) and in age-adjusted analyses. Gestational diabetes was consistently highest among females greater than 35 years old and those in the lowest SES category. The annual percent change (APC) between 1981-2009, prior to the inflection point, was 1.93% (95% CI: 1.39-2.48%) and was 11.7% (95% CI: 8.87-14.7%) post inflection – from 2010-2019. After 2010, gestational diabetes incidence increased most among urban residents (APC= 18.1%; 95%CI: 13.9-22.5), among those >35 years (APC = 12.00%; 95% CI: 8.45-15.70%), and among individuals in the

highest SES group (APC = 14.8%; 95% CI: 9.39-20.4%).Geospatial mapping showed that gestational diabetes incidence increased more in neighbourhoods with the highest proportion of recent immigrants to Canada.

Conclusion: Annual incidence of gestational diabetes increased six-fold in Manitoba, over the last 20 years particularly among those with high SES and higher maternal age. However, this may be partially due to a change in screening practices as evidenced by the upward inflection consistent across strata.

Introduction

Diabetes is the most common metabolic perturbation in pregnancy worldwide.[121, 127] Of all forms of diabetes in pregnancy, gestational diabetes – elevated blood glucose in the second or third trimester[128] – is the most common.[129] Pregnancies complicated by gestational diabetes are associated with other disorders of pregnancy (e.g., pregnancy-induced hypertension), birthing complications (e.g., caesarean delivery and preterm delivery), and poorer outcomes for newborns (e.g., macrosomia and poor Apgar scores).[130-132] Females with gestational diabetes are also at greater risk of developing type 2 diabetes and cardiovascular disease later in life,[133-135] and there may be long-term health implications for exposed offspring including obesity, diabetes, and cardiometabolic disease.[136, 137]

Given the perinatal complications associated with gestational diabetes and the risk for long- and short-term adverse health outcomes, monitoring trends over time is a public health priority, especially among high risk groups.[138] In Manitoba, gestational diabetes increased steadily from 1984 – 2004 and was consistently higher among those aged 35 or older and those

living in rural areas.[126] However, it is unclear to what extent these trends continued and how they differed between various high-risk groups.

Individual level data on factors that contribute to persistent health inequities (e.g., ethnicity, and immigration status) are often not available within administrative health databases that are used for monitoring disease. Geospatial mapping provides a method for tracking disease trends in physical space where postal codes are converted into geographic coordinates allowing for visualization of disease by neighbourhood area. Demographic information from the national census can be linked to neighbourhood areas providing novel insight into disease rates among sub-populations experiencing inequities. To the best of our knowledge, geospatial mapping has not been used to describe trends in gestational diabetes across populations.

We relied on population-based administrative health data and geospatial mapping of the province's largest urban centre to describe trends and inequities in annual gestational diabetes incidence in Manitoba, Canada from 1981 to 2019. The primary study objectives were to (1) describe overall provincial trends in gestational diabetes incidence, (2) describe incidence among sub-groups of females that often experience health inequities in Manitoba, and (3) apply geospatial mapping to describe gestational diabetes incidence across census dissemination areas in a large urban centre.

Methods

Study design

We conducted a descriptive study of trends in annual incidence of gestational diabetes using time-series data collected form a population-based administrative health data repository.

We leveraged administrative health, social and census data housed at the Manitoba Centre for Health Policy (MCHP) using nine-digit numeric personal health identification numbers that have been encrypted to preserve anonymity.[139] De-identified IDs were used to link person level data across multiple datasets including Manitoba Health Insurance Registry, Hospital Abstracts, Medical Claims, and the Drug Program Information Network File. Canada Census data at the dissemination area level was assigned to individuals based on their postal code of residence via the Postal Code Conversion File. Through these linkages we were able to create a unique dataset that was comprised of virtually all pregnant people in Manitoba who accessed the provincial healthcare system between 1981 – 2019.[140] The efficacy and validity of data housed at MCHP has been described previously.[141, 142] This study was approved by the Health Research Ethics Board at the University of Manitoba (HREB: #HS20928; H2017:232) and the provincial Health Information Privacy Committee (HIPC No. 2019/2020-05). We followed STROBE and RECORD reporting guidelines (Chapter 10, subsection 10.1)[143] and recommendations for reporting descriptive studies.[144]

Population and outcomes

The final dataset consisted of females aged 18 to 50 years who had a live-birth or still-birth delivery between April 1, 1981 to March 31, 2019 and had three or more years of healthcare coverage in total. We excluded those with a diagnosis of pre-existing diabetes, defined as having two or more diagnosis codes for type 1 or type 2 diabetes within three years of each other, a prescription for insulin pump supplies, or a prescription for antihyperglycemic medications with at least 1 diagnosis of diabetes (Figure 4.1).

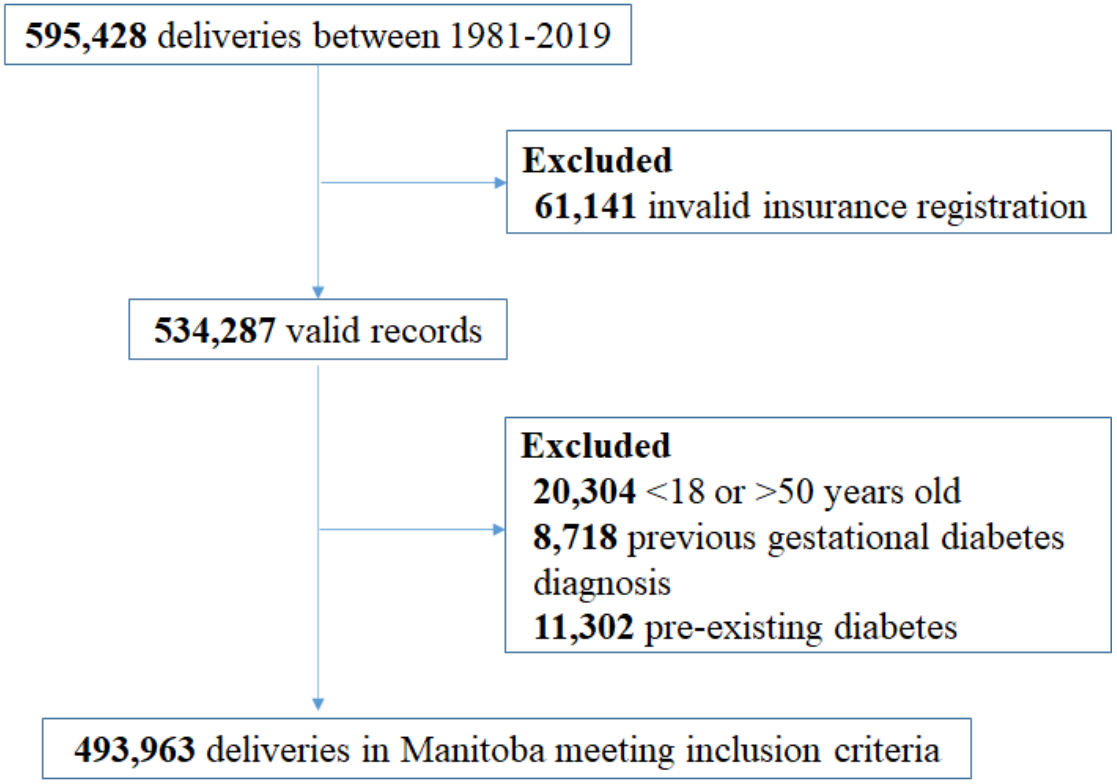

Figure 4.1 Participant cohort flow chart

Gestational diabetes defined as having received one or more hospitalization codes with a diagnosis of ICD-9-CM 648.8 or ICD-10-CA O24 at 21 weeks of gestation or greater with no prior diagnosis for pre-existing diabetes.[145-149] Individuals with a diagnosis of diabetes <21 weeks of gestation were considered as having pre-existing diabetes[150] and were excluded from the analyses.

The secondary outcome of interest, used in a sensitivity analysis, was incidence of large for gestational age offspring born to women in the cohort. We included large for gestational age as an outcome as it is both a well-recognized complication of gestational diabetes and treatment of gestational diabetes reduces its risk.[151-154] Therefore, if gestational diabetes is increasing, we would expect to see an increase in large for gestational age infants. If gestational diabetes diagnosis is becoming more sensitive (i.e., diagnosis occurring at a lower blood sugar level), we

would expect rates of large for gestational age to decline. The lambda-mu-sigma method was used to calculate z scores from birthweight derived from population-based references according to the Fenton method.[155] large for gestational age was defined as a birthweight above the 90th percentile for gestational age and sex.[156]

Covariates

We selected multiple covariates to define sub-groups of females that often experience health inequities. Maternal age at time of delivery was stratified into three sub-groups: 18-25, 26-35, and >35years. We also stratified by urban and rural residence, defined using maternal postal code at delivery. Those living outside the two major urban cities in Manitoba were classified as rural. Gestational diabetes incidence was also assessed by income quintile, defined as mean neighbourhood household income based on the delivery postal code. Income quintiles were considered separately for rural and urban groups because of the difference in population density and income distribution in Manitoba. Additionally, we created four sub-groups based on Socioeconomic Factor Index – Version 2 (SEFI-2) which is a proxy measure for neighbourhood-level social and material deprivation. SEFI-2 is a continuous variable with a standard normal distribution that is derived from Census variables including: unemployment rate, average household income, proportion of single parent households, and proportion of population without high school graduation.[157] Higher SEFI-2 reflects higher degree of deprivation. Trends in gestational diabetes were also compared by Regional Health Authority (RHA) which represents governmental areas responsible for health service delivery for specific geographical areas in Manitoba.[158] Geospatial mapping was used to describe gestational diabetes trends within neighbourhood dissemination areas in the city of Winnipeg, the provincial capital and largest city in the province.

Analyses

Incident gestational diabetes was calculated as the first case of gestational diabetes per person. Subsequent cases were not included as mothers with a history of gestational diabetes are at higher risk of developing diabetes in future pregnancies.[159] We calculated age-standardized rates of gestational diabetes using the 2016 cohort of deliveries as the standard group. The annual percent change in gestational diabetes was calculated using a segmented Poisson regression to assess trends over time for the entire province and among subgroups. Confidence intervals were derived using bootstrapping.

To conduct geospatial mapping, we used the geographic information system software ArcGIS by Environmental Systems Research Institute to describe changes in rates of gestational diabetes across dissemination areas in Winnipeg, Canada. Winnipeg is the largest city in Manitoba, accounting for over half of the provinces' population. The definitions and boundaries for dissemination areas used for geospatial mapping were adopted from those used in the Canadian Census. Data from the 2016 census were used to describe demographic characteristics of the neighbourhood dissemination areas.

Results

A total of 595,428 deliveries were recorded between 1981 and 2019. After excluding those that did not meet inclusion criteria, there were 493,963 deliveries at risk for gestational diabetes (Figure 4.1). Total deliveries per year remained relatively stable in Manitoba between 1981 - 2019 (48 ± 4 deliveries per year per 1000 females) with a slight downward trend (52 vs 45 deliveries per 1000 females in 1981 vs 2019, respectively). Most deliveries occurred among individuals living in urban centres (n=277,602; 56.2%), individuals aged 25-36 years

(n=276,721; 56.0%) at the time of delivery, and individuals in the lowest income quintiles (Table 4.1).

Crude cumulative incidence of gestational diabetes in Manitoba between 1981 – 2019 was 3.1% (n = 15,258). Gestational diabetes incidence increased from 1.32% in 1981 to 8.65% in 2019 with an inflection beginning around 2010 (Figure 4.2). The average annual percent change in gestational diabetes incidence for the entire duration of the study (1981 – 2019) was 3.97% (95% CI: 1.06-7.00%), The average annual percent change between 1981-2009, prior to the inflection point, was 1.93% (95% CI: 1.39-2.48%) and was 11.7% (95% CI: 8.87-14.7%) post inflection – from 2010-2019. Trends across sub-groups paralleled province-wide trends between 1981 and 2019 in that there was a noticeable inflection that occurred around 2010 in all groups (Figure 4.3). Over the 38-year period gestational diabetes incidence was consistently higher in the highest age group, the Northern Health Region, the lowest socioeconomic group, and the lowest rural income quintiles (Figure 4.3A, B, C, and E). Individuals with higher parity also had a consistently higher risk of gestational diabetes; however, this trend was attenuated after adjusting for age at delivery (data not shown. There were no other notable differences between age-standardized and crude incidence among any of the other sub-groups (data not shown).

Table 4.1 Delivery characteristics by diabetes status

	Gestational Diabetes	**No Diabetes**	**Total**
Total Deliveries	**n (%)**	**n (%)**	**n (%)**
1981 – 2019	15,258 (3.1)	478,705 (96.9)	493,963 (100)
Maternal Characteristics 1981 - 2019			
Age (years)			
18 - 25	3,989 (26.1)	172,581 (36.1)	176,570 (35.7)
26 - 35	8,540 (56.0)	268,180 (56.0)	276,720 (56.0)
>35	2,729 (17.9)	37,944 (7.9)	40,673 (8.2)
Parity			
Nulliparity	5606 (36.8)	174,392 (36.7)	179,998 (36.7)
1	4376 (28.7)	162,398 (34.2)	166,774 (34.0)
2	2365 (15.5)	79,138 (16.7)	81,503 (16.6)
≥3	2882 (18.9)	59,040 (12.4)	61,922 (12.6)
Residence			
Rural	6,642 (43.5)	209,719 (43.8)	216,361 (43.8)
Urban	8,616 (56.5)	268,986 (56.2)	277,602 (56.2)
Income Quintile			
Rural			
R1 (lowest)	2,607 (39.3)	50,597 (24.1)	53,204 (24.6)
R2	1,318 (19.8)	41,221 (19.7)	42,539 (19.7)
R3	892 (13.4)	38,642 (18.4)	39,534 (18.3)
R4	933 (14.0)	41,330 (19.7)	42,263 (19.5)
R5 (highest)	868 (13.1)	37,598 (17.9)	38,466 (17.8)
Not Found	24 (0.4)	331 (0.2)	355 (0.2)
Urban			
U1 (lowest)	2,509 (29.1)	63,544 (23.6)	66,053 (23.8)
U2	1,976 (22.9)	58,170 (21.6)	60,146 (21.7)
U3	1,592 (18.5)	52,965 (19.7)	54,557 (19.7)
U4	1,459 (16.9)	51,681 (19.2)	53,140 (19.1)
U5 (highest)	1,061 (12.3)	41,736 (15.5)	42,797 (15.4)
Not Found	19 (0.2)	890 (0.3)	909 (0.3)
Regional Health Authority			
Interlake-Eastern	1,512 (9.9)	43,777 (9.1)	45,289 (9.2)
Northern Health Region	2,687 (17.6)	45,962 (9.6)	48,649 (9.8)
Southern Health-Sante Sud	1,406 (9.2)	77,456 (16.2)	78,862 (16.0)
Prairie Mountain Health	1,775 (11.6)	61,816 (12.9)	63,591 (12.9)
Winnipeg Regional Health Authority	7,878 (51.6)	249,694 (52.2)	257,572 (52.1)
SEFI Category			
< -1 (highest)	1,246 (8.2)	59,597 (12.4)	60,843 (12.3)
-1 – 0	4,927 (32.3)	180,348 (37.7)	185,275 (37.5)
0 – 1	4,576 (30.0)	151,219 (31.6)	155,795 (31.5)
>1 (lowest)	4,509 (29.6)	87,541 (18.3)	92,050 (18.6)

SEFI, Socioeconomic Index Factor – Version 2. A higher SEFI score represents greater deprivation (i.e., lower socioeconomic status).

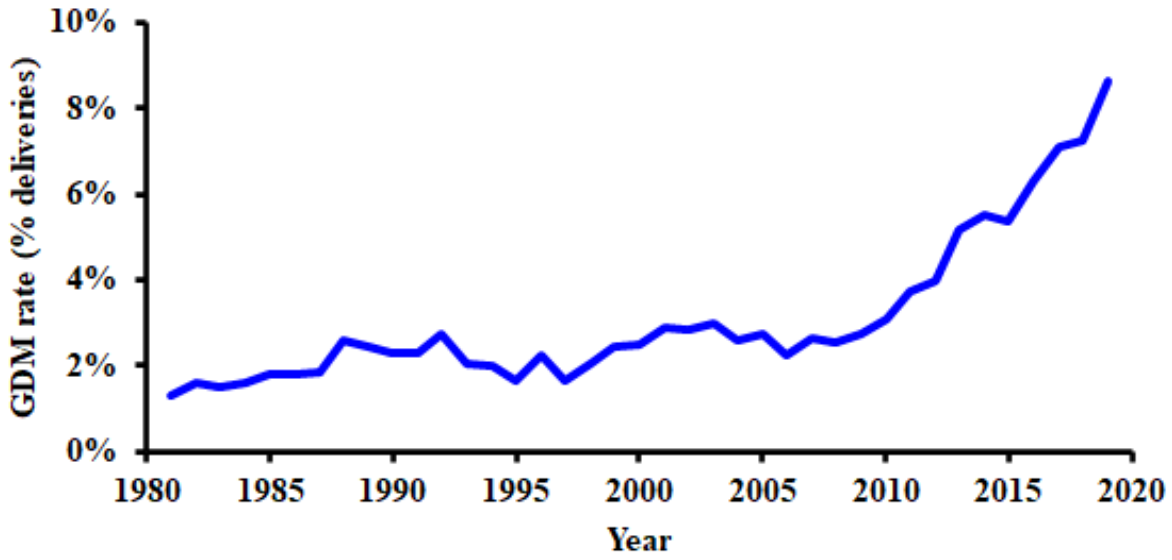

Figure 4.2 Incidence of gestational diabetes by year in Manitoban's from 1981-2019.

The annual percent change increased among all subgroups by at least 4 percentage points before and after the inflection that occurred after 2010 (Table 4.2). The highest overall annual percent change in gestational diabetes incidence occurred during the post-inflection period in the highest urban income quintile (18.1%; 95%CI: 13.9-22.5) and this group also had the greatest difference between the pre-inflection and post-inflection periods (17.03 percentage points). The highest rural income quintile also had a greater annual percent change of gestational diabetes after the inflection and greater difference pre- to post-inflection compared to the other rural income quintiles. Similarly, when stratified by SEFI-2, the higher socioeconomic groups had the highest annual percent change in gestational diabetes incidence following the inflection (i.e., 2010 -2019) and the greatest increase compared to the pre-inflection period.

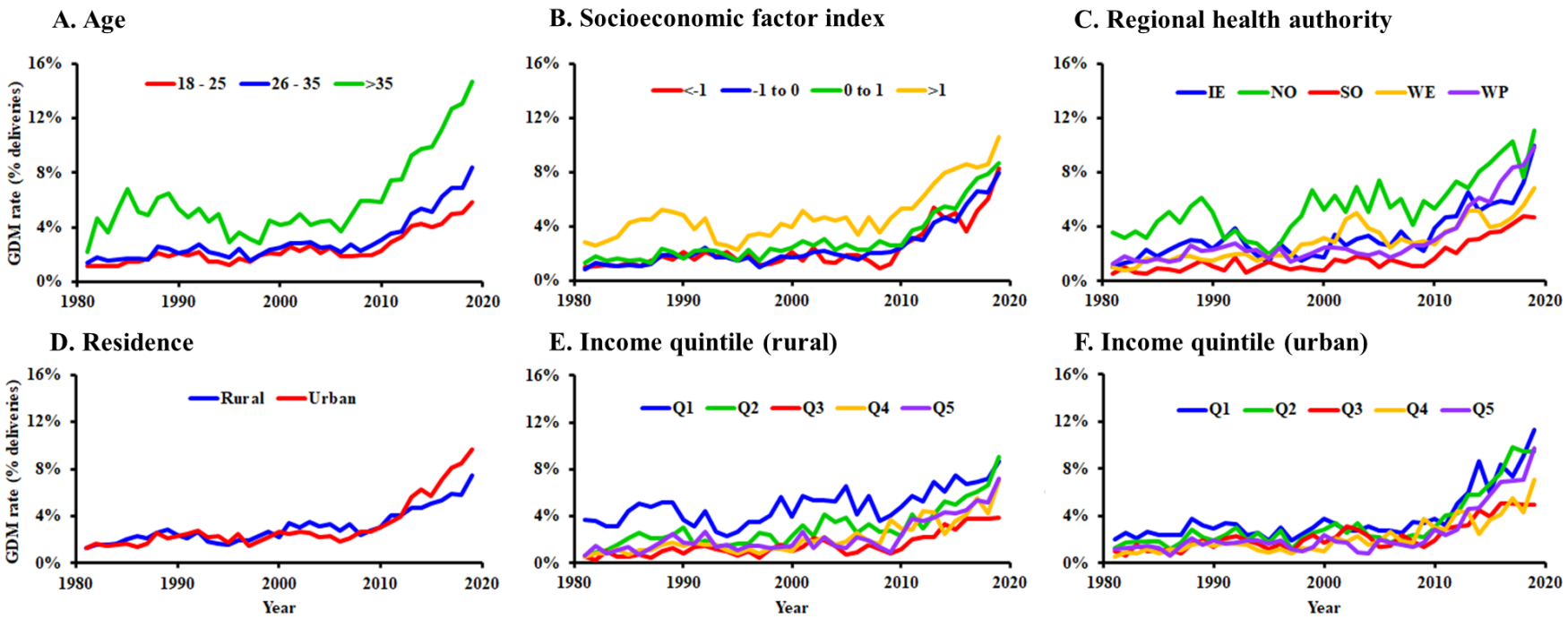

Figure 4.3 GDM incidence by calendar year from 1981 – 2019. GDM, gestational diabetes mellitus; IE, Interlake-Eastern; NO, Northern Health Region; SO, Southern Health-Santé Sud; WE, Prairie Mountain Health; WP, Winnipeg Regional Health Authority.

Table 4.2 Annual percent change in gestational diabetes incidence before and after inflection.

	Annual Percent Change (95% CI)		
	1981-2009	2010-2019	Δ
Age (years)			
18 - 25	1.89 (1.24-2.54)	9.53 (6.22-13.0)	7.64
26 - 35	1.65 (1.11-2.20)	11.50 (8.68-14.4)	9.85
>35	0.12 (-0.63-0.88)	12.00 (8.45-15.70)	11.88
Parity			
Nulliparity	1.95 (1.28-2.63)	12.7 (9.21-16.2)	10.75
1	2.16 (1.63-2.70)	11.9 (9.35-14.6)	9.74
2	1.65 (0.96-2.34)	11.4 (8.08-14.8)	9.75
≥ 3	0.31 (-0.52-1.14)	8.70 (4.48-13.1)	8.39
Residence			
Rural	2.07 (1.41-2.72)	7.98 (4.70-11.40)	5.91
Urban	1.62 (0.96-2.29)	14.3 (10.8-18.0)	12.68
Income Quintile			
Rural			
R1 (lowest)	0.85 (0.05-1.66)	5.07 (1.11-9.19)	4.22
R2	2.48 (1.48-3.49)	9.09 (4.54-13.8)	6.61
R3	2.34 (1.37-3.32)	9.18 (4.89-13.6)	6.84
R4	3.77 (2.61-4.95)	9.05 (3.89-14.5)	5.28
R5 (highest)	1.73 (0.60-2.87)	13.4 (7.89-19.1)	11.67
Urban			
U1 (lowest)	0.82 (0.08-1.56)	13.2 (9.48-17.1)	12.38
U2	1.47 (0.62-2.33)	15.0 (10.7-19.5)	13.53
U3	2.93 (1.95-3.92)	11.7 (7.05-16.5)	8.77
U4	1.99 (0.96-3.02)	14.7 (9.51-20.1)	12.71
U5 (highest)	1.07 (0.16-1.99)	18.1 (13.9-22.5)	17.03
Regional Health Authority			
Interlake-Eastern	1.69 (0.67-2.71)	10.6 (5.59-15.9)	8.91
Northern Health Region	1.44 (0.51-2.37)	5.77 (1.16-10.6)	4.33
Southern Health-Sante Sud	2.48 (1.60-3.37)	13.0 (9.07-17.0)	10.52
Prairie Mountain Health	3.97 (3.02-4.94)	4.10 (-0.19-8.58)	0.13
Winnipeg Regional Health Authority	1.33 (0.62-2.05)	15.6 (11.7-19.6)	14.27
SEFI Category			
< -1 (highest)	1.44 (0.43-2.46)	14.8 (9.39-20.4)	13.36
-1 – 0	1.94 (1.23-2.65)	13.7 (10.1-17.4)	11.76
0 – 1	2.13 (1.60-2.67)	12.2 (9.63-14.9)	10.07
>1 (lowest)	0.64 (-0.11-1.39)	8.78 (4.93-12.8)	8.14

SEFI, Socioeconomic Index Factor – Version 2.

Geospatial mapping across census dissemination areas in Winnipeg, Manitoba allowed us to describe characteristics of neighbourhoods with the lowest and highest risk of gestational diabetes over the last decade, after the upward inflection of cases (2010-2019). The neighbourhoods that had the highest incidence of gestational diabetes also had the highest proportion of racialized persons and the highest proportion of those who immigrated in the last ten years (Figure 4.4, Table 9.1). Conversely, the neighbourhood areas with the lowest gestational diabetes incidence had the lowest proportion of racialized persons and the lowest proportion of Canadian newcomers (Figure 4.4; Table 9.1).

In contrast to the incidence of gestational diabetes, incidence of large for gestational infants among all births decreased by 3.1 percentage points between 2000 - 2019. Unlike gestational diabetes, trends in large for gestational age infants were characterised by a downward inflection that occurred around 2010 (Figure 9.1). When stratified by diabetes type, there was a 12.3 percentage point decrease in incidence of large for gestational age infants among individuals with gestational diabetes and a 3.6 percentage point decrease in large for gestational age incidence among pregnancies complicated by pre-existing diabetes.

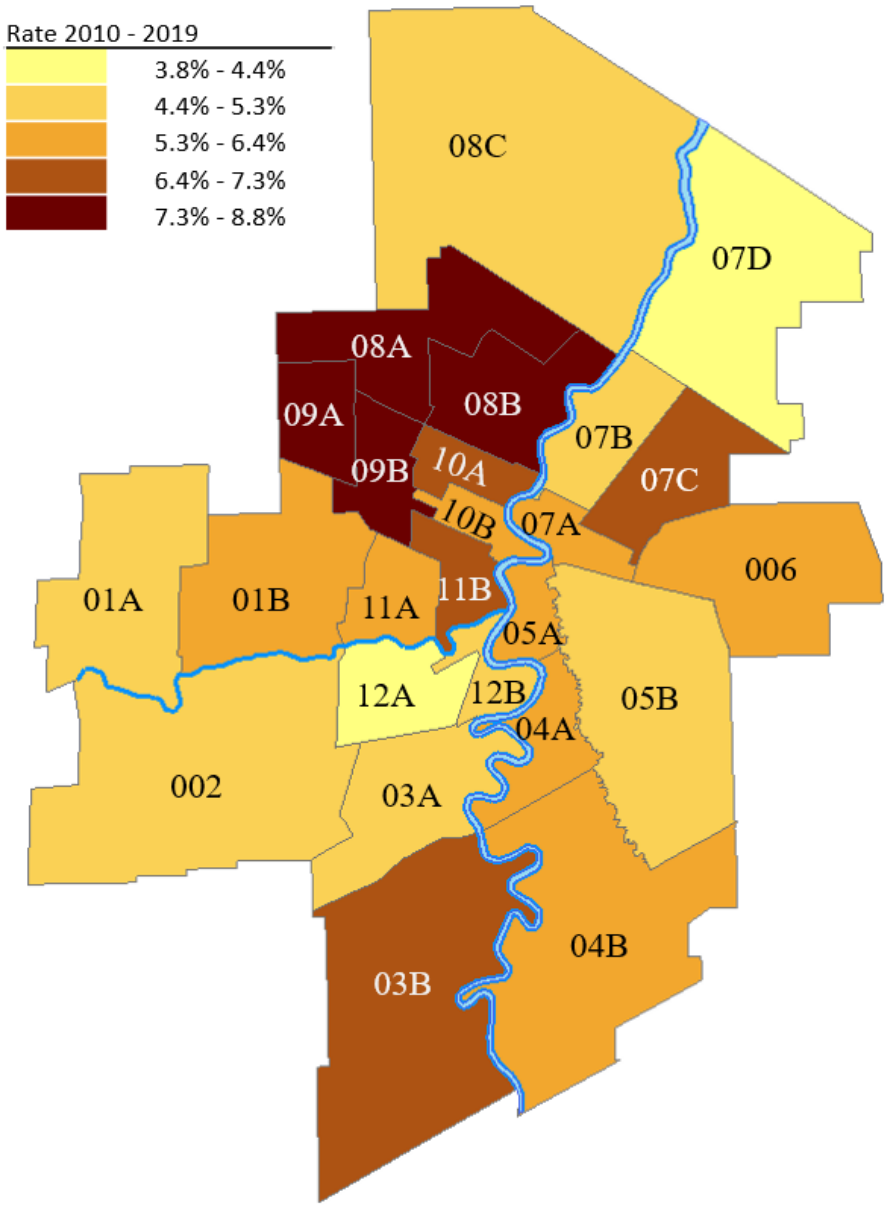

Figure 4.4. Gestational diabetes incidence by Winnipeg neighbourhood dissemination area from upward inflection (2010) until end of study (2019).

Discussion

The main finding from this descriptive population-based study was that between 1981 and 2019 gestational diabetes increased 6-fold in the province of Manitoba with a distinct upward inflection occurring around 2010. Gestational diabetes incidence was consistently

highest among individuals experiencing the highest levels of economic and social deprivation, those living in Northern and rural areas, and those over 35 years old. However, the greatest change over time occurred among those living in urban centers and individuals with less social deprivation and higher income. Within the largest urban centre in Manitoba, incidence of gestational diabetes was consistently highest in neighbourhoods characterized by the highest proportion of racialized individuals and recent immigrants to Canada. Finally, incidence of large for gestational age infants declined over time, particularly among infants born to females with gestational diabetes.

The increased incidence of gestational diabetes observed across the population is consistent with trends observed in other provinces in Canada.[123, 124, 160] Similar to others, we found disproportionately higher incidence of gestational diabetes among those living in areas with high social and material deprivation,[124] lower income neighbourhoods,[161] and those 35 years of age.[160] Higher maternal age is a well-established risk factor for gestational diabetes,[132, 162] though the highest age group (age greater than 35 years) made up a relatively small proportion (8.2%) of the entire cohort (Table 4.1). This likely explains why age-standardized analyses were unremarkable. Similar to other groups we documented that gestational diabetes incidence was persistently higher among individuals from lower socioeconomic and income groups.[157, 163-165] The Northern Health Region has a considerably lower population density compared to the entire province of Manitoba (0.18 vs 2.19 persons per km^2, respectively), a large number of smaller towns and communities, and a population where over two-thirds of residents self-identify as Indigenous. Therefore, the consistently higher incidence of gestational diabetes in the Northern Health Region may represent additional structural health inequities such as poorer or variable

healthcare service delivery and ongoing impacts of colonization for Indigenous people living in those areas.[166, 167]

In addition to higher incidence of gestational diabetes in the northern region of the province, Geospatial mapping data revealed a disproportionate rise in gestational diabetes in urban neighbourhoods characterized by higher proportions of recent immigrants and racialized individuals. These data are in line with what others have shown,[168, 169] and may reflect differences in screening rates, community outreach, and clinical practice for pregnant people within these sub-groups. Additional studies with individual race and ethnicity data for pregnant individuals are needed to confirm these observations.

The documented inflection in the annual incidence of gestational diabetes around 2010 described here is likely partially explained by changes in screening practices.[125, 170] A recent study conducted in British Columbia, Canada found that roughly half of the increase in gestational diabetes incidence in the province was related to the 2010 changes in screening methods.[125] Others have documented that changes in screening practices, where the criteria to diagnose gestational diabetes is more sensitive, contribute to rising rates of gestational diabetes.[170] We were not able to measure screening practices in the current study, so it is unclear to what extent changes to screening practices contributed to the trends we described here. However, given the comparably larger increases in the annual percent change of gestational diabetes in typically low-risk groups (i.e., low socioeconomic deprivation, high income, and living in an urban area) following the 2010 inflection, it is likely that changes in screening disproportionately affected these groups. Others have reported favourable perinatal outcomes following changes in screening practices[171, 172] which may be consistent with our findings of the reduction in the proportion of large for gestational age infants among gestational diabetes

pregnancies. This decrease in large for gestational age infants, a known complication of gestational diabetes pregnancies, could suggest that less severe forms of diabetes are being diagnosed and therefore there are fewer pregnancies with large for gestational age infants as a result. The etiology underlying the decreasing proportion of large for gestational infants being delivered in gestational diabetes pregnancies are likely multifactorial, warranting further investigation into the causes of these compelling trends in gestational diabetes incidence and perinatal outcomes in Manitoba.

Limitations and conclusion

The study is strengthened by the use of a population-based dataset of females with data available over a 30 year period. However, as with all administrative database studies, there is a risk of misclassification bias due to the use of data not collected specifically for research purposes. Prescription data prior to 1995 are unavailable in MCHP, therefore, a small number (~1.2%) of individuals with type 1 or type 2 diabetes might have been misclassified as having gestational diabetes and included in disease estimates. Additionally, we did not have access to data prior to 1981 to exclude persons who had a previous gestational diabetes-affected delivery so gestational diabetes incidence may be slightly overestimated in the first few years of the analysis. However, due to the robust trends seen here, it is unlikely that misclassification bias would significantly change our estimates. Lastly, in the absence of individual race and ethnicity data, we were unable to specifically describe trends in gestational diabetes based on sub-groups that experience health inequities and instead used neighbourhood level data as a proxy. Despite these limitations, it is clear that gestational diabetes incidence is increasing in Manitoba and is consistently, disproportionately occurring among individuals in lower SES groups, with higher

maternal age, and living in urban areas with higher proportions of racialized individuals and newcomers to Canada.

Acknowledgements

The authors acknowledge the Manitoba Centre for Health Policy for use of data contained in the Manitoba Population Research Data Repository. The results and conclusions are those of the authors and no official endorsement by the Manitoba Centre for Health Policy, Manitoba Health, or other data providers is intended or should be inferred. Data used in this study are from the Manitoba Population Research Data Repository housed at the Manitoba Centre for Health Policy, University of Manitoba and were derived from data provided by Manitoba Health and the Winnipeg Regional Health Authority. The final draft of the manuscript was sent to the data providers (the Health Information Privacy Committee [HIPC] and the Winnipeg Regional Health Authority) regarding confidentiality, privacy and consistency with the HIPC-approved protocol, but they had no control on the results presented or the decision to submit the manuscript for publication. We heartfully thank Mr. Charles Burchill for his logistical support in cutting the required data and advice for analysing it.

Chapter 5: Exposure to Gestational Diabetes in Utero and Hypertension in Offspring: A Triangulation Approach

Authors: Nicole Brunton, Allison Dart, Nathan Nickel, Heather Prior, Todd A. Duhamel, Jonathan McGavock

Manuscript status: To-be submitted.

5.1 Contribution of authors

I developed this project with support from Dr. McGavock. I wrote the proposal and received additional input from Dr. Dart, Dr. Nickel, and Dr. Duhamel. I sought the necessary approvals from MCHP, the University of Manitoba Research Ethics Board, the Health Information Privacy Committee, and the Winnipeg Regional Health Region. Mrs. Heather Prior and Mr. Randy Walld (MCHP analysts) supported me in cleaning the raw data obtained from MCHP and creating the birth cohorts. I completed all analyses with support from Mrs. Heather Prior who inspected my programs for quality assurance. I interpreted the results, wrote the manuscript, and created the tables and figures. Drs. McGavock, Dart, Duhamel, and Nickel provided valuable feedback throughout the planning, execution, and writing of the manuscript.

5.2 Preface to manuscript

As see in the previous chapter, the incidence of gestational diabetes is increasing and, as described in Chapter 2, this could translate into worsening health for offspring due to the association between exposure to diabetes in utero and elevated risk of high blood pressure and hypertension in those offspring. However, it is unclear whether the observed association

represents a causal in-utero mechanism. The following manuscript describes the study conducted to address this knowledge gap.

5.3 Manuscript

Abstract

Background: Several observational studies suggest that exposure to gestational diabetes is associated with increased risk of hypertension among offspring. It is hypothesized that this association represents a causal relationship.

Methods: This study leveraged population-level administrative health data from 418,169 singleton born offspring from 224,963 families. Gestational diabetes and adolescent hypertension were determined using validated ICD code-based algorithms. Three complimentary analyses were used to test the main hypothesis. First, we compared rates of hypertension between offspring exposed to gestational diabetes and controls matched on birthyear, socioeconomic index, maternal age, parity, residence, and health region using a Poisson model with person-years of the denominator as an offset. Second, we repeated these analyses using paternal type 2 diabetes status as a negative exposure control. Lastly, a sibling analysis compared the incidence of hypertension between siblings discordant for gestational diabetes exposure.

Results: Between unrelated offspring (n=209,417), the prevalence of youth-onset hypertension among all offspring was 3.0%. The incident rate of hypertension was 35% higher among offspring exposed to gestational diabetes compared to unexposed offspring (IRR:1.35, 95%CI: 1.19,1.54). The IRR was similar after matching on covariates (n=11,612; IRR:1.41, 95%CI: 1.16,1.72). Within the cohort of all paternal-offspring pairs (n=145,298), hypertension rate was

1.7-fold higher among offspring whose fathers lived with diabetes in unadjusted analyses (IRR:1.70, 95% CI: 1.28,2.26) and 1.5 times higher after exact matching (n=2,022; IRR:1.49, 95%CI: 0.95,2.33). Finally, incidence of youth-onset hypertension was similar among sibling pairs discordant for exposure to gestational diabetes (n=12,204; RR:1.02, 95%CI: 0.85,1.22).

Conclusion: The observed association between exposure to gestational diabetes and elevated hypertension rate is unlikely to be driven by a direct in utero mechanism.

Introduction

Observational studies suggest that complex chronic diseases like hypertension may have their etiological origins in utero. For example, several investigations into the metabolic abnormalities that increasingly complicate pregnancies[84] have consistently reported associations between hyperglycemia,[85] pre-pregnancy diabetes,[12] and gestational diabetes[11] and various markers of adverse cardiovascular health in offspring, including increased blood pressure and hypertension.[173] However, there are a some studies that contradict these findings[174, 175] and, over the past decade, studies applying novel methods have provided conflicting evidence of a direct intrauterine mechanism leading to altered offspring development causing chronic diseases, like hypertension.[97, 98] Therefore, whether a direct causal relationship exists between exposure to gestational diabetes in utero and increased risk of hypertension in the offspring remains unclear.

Researchers face various methodological challenges when trying to elucidate causal links between in-utero exposures and long-term health outcomes in offspring. For example, randomized controlled experiments are often ethically or practically unfeasible, large prospective cohort studies are often required, and data on important confounding variables are not always available.[176] One strategy to overcome these limitations is the application of triangulation.

Etiological triangulation is an emerging method that can improve our ability to infer causation from observational data by simultaneously interpreting evidence from multiple studies that compliment each other in terms of bias, assumptions, and limitations.[117] Interpreting results from three distinct observational studies, with different sources of bias, can address causal ambiguity surrounding associations between in-utero exposures and long-term health outcomes in offspring.[120, 177]

We used an etiological triangulation approach to test the hypothesis that exposure to diabetes during pregnancy is a cause of elevated hypertension in offspring. Three unique prospective cohort study designs were used to test this hypothesis including (1) a prospective exact-matched study, (2), a paternal negative exposure control study, and (3) a discordant sibling-matched study (Figure 5.1).[120]

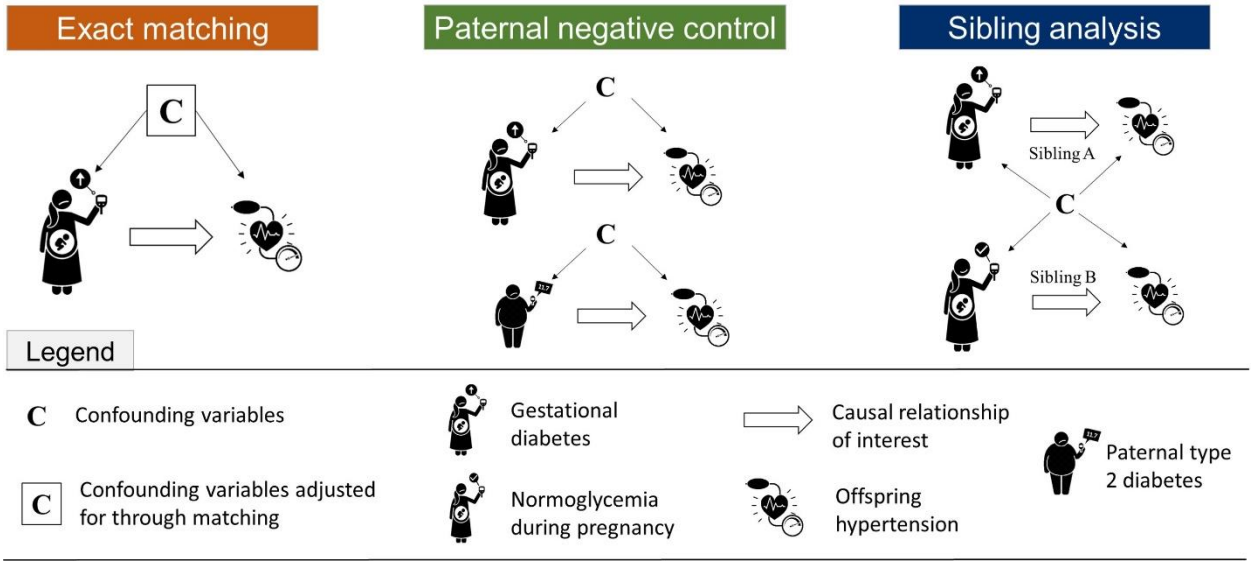

Figure 5.1 Overview of three study designs used to assess the association between exposure to gestational diabetes an offspring risk of hypertension in adolescence. Figure adapted from Borges et al. (2024).[116]

Methods

Population

For each study we leveraged administrative health data housed at the Manitoba Centre for Health Policy data repository within the University of Manitoba. The data repository contains linkable administrative records for all registered Manitobans. All data are de-identified (i.e., personal identifiers such as names and addresses are removed) and encrypted nine-digit personal health identification numbers are used to preserve anonymity.[139] As done previously, the de-identified personal health numbers were used to link data across several datasets including: the Manitoba health insurance registry, hospital discharge abstracts, Canadian Census public use file, Diabetes Education Resource for Children and Adolescents database, the Drug Program Information Network, and Medical Claims (Table 9.3).[178] The efficacy and validity of these administrative data have been described previously.[141, 142] This study was approved by the Health Research Ethics Board at the University of Manitoba (HS19742; H2016:185) and the provincial Health Information Privacy Committee (HIPC No. 2016/2017-06). The STROBE checklist was used to guide reporting for each study (Chapter 10, subsection 10.2).[143]

We created a cohort of liveborn singletons born between April 1, 1979 - March 31, 2011, who were followed until March 31, 2021. This ensured that all offspring included in the study reached at least 10 years of age by the latest follow-up as the largest increases in blood pressure occur during adolescence[179] which is defined by the World Health Organization as age 10 – 19 years. We excluded anyone who died during their birth hospitalization, were preterm (<37 weeks), had very low birthweight (<500g), or had invalid insurance registration. Individuals were also excluded if that had any of the following diagnoses: congenital anomaly of the circulatory system, endocardial fibroelastosis, endomyocardial disease, congenital cardiomyopathy, or cystic fibrosis (Table 9.4). Individuals were linked to their mother and those

who could not be linked were excluded. Through this process we created a dataset of 418,169 singletons born from 224,963 unique mothers. Of the 418,169 births, 259,303 were linked, through the Manitoba Health Registry, to a male family head (62%) who was identified as the father. There were 147,836 unique fathers identified. See Figure 9.2 for a detailed flowchart of participants included in study cohorts.

Exposures of interest

The primary exposure was diabetes during pregnancy. We assessed three exposures of interest including gestational diabetes, type 2 diabetes in mothers, and type 2 diabetes in fathers (a negative exposure). We used validated algorithms based on ICD codes and prescription data to classify pregnant persons as having gestational or type 2 diabetes.[147-149] Gestational diabetes was defined as a diagnosis of diabetes between 21 weeks gestational and 6 weeks post partum. Type 2 diabetes was defined in accordance with previously used definitions.[180] Briefly, an individual was considered to have type 2 diabetes if they had received a diagnosis code on one or more hospitalizations or two or more physician visits within two years. If they had one or more prescriptions to treat diabetes, one or more prescriptions for metformin in combination with one physician visit, one or more hemoglobin A1C tests $\geq$ 6.5%, or were identified as having type 2 diabetes in childhood or adolescence within the Diabetes Education Resource for Children and Adolescence database (Table 9.3). It was important to include maternal Type 2 diabetes as an exposure to consider timing and whether exposure to diabetes earlier in pregnancy (i.e., before 21 weeks) conferred additional risk of hypertension among offspring. Type 2 diabetes was defined the same for mothers and fathers. If an individual identified as having type 2 diabetes met any of the criteria for type 1 diabetes, they were considered to have type 1 diabetes. All individuals with type 1 diabetes were excluded from all analyses due to the distinct etiology of

type 1 diabetes and unique set of complications. For details on ICD codes and disease definitions, see Table 9.4 and Table 9.5 in the supplementary material (Chapter 9).

Outcome

The primary outcome for all analyses was youth-onset hypertension in offspring, defined as a diagnosis at or before age 19 years. Hypertension diagnosis in youth was chosen to increase proximity to the exposure and because previous work from our lab demonstrated an increased risk of developing a cardiovascular end point before age 20 among offspring exposed to diabetes in utero.[178] We followed a validated method for defining hypertension in youth within an administrative health database.[59] Briefly, an individual was considered to have hypertension if they had one or more hospitalizations or physician claims with a diagnosis code for hypertension (Table 9.4 and 9.5) or at least one prescription for an anti-hypertensive medication (Table 9.6).

Incident rate of hypertension among all offspring was calculated as the number of new cases of hypertension diagnosed over the study period divided by person-years at risk. Time at risk was calculated from birth and an individual was no longer considered at risk once they received a diagnosis for hypertension (as per our definition above), turned 19, died, moved out of the province, or reached the end of the study (March 31, 2021). All offspring were included in primary analyses, regardless of number of years of follow-up. In sensitivity analyses, inclusion was restricted to offspring who had turned 19 by the end of the study and had not died or moved out of the province.

Covariates

Potential confounding variables that are adjusted for in all models included: maternal age, birth year, urbanicity, Regional Health Authority, and socioeconomic deprivation. All variables were taken at the time of offspring's birth to avoid inadvertently adjusting for mediating variables. Parental age at time of delivery was left as a continuous variable in all models. Urbanicity was categorized as urban and rural residence, defined using the parent's postal code at delivery. Those living outside the two major urban cities in Manitoba were classified as rural. Socioeconomic deprivation was measured using the Socioeconomic Factor Index – Version 2 (SEFI-2) which is a proxy measure of neighbourhood-level social and material deprivation. SEFI-2 is a continuous variable with a standard normal distribution that is derived from Census variables including: unemployment rate, average household income, proportion of single parent households, and proportion of population without high school graduation.[157] Higher SEFI-2 reflects higher degree of deprivation. We also adjusted for Regional Health Authority which represents governmental areas responsible for health service delivery for specific geographical areas with the province.[158] We also adjusted for parity at time of offspring birth in maternal-offspring analyses. All adjusted confounding variables were derived from the MCHP databases listed above and in Table 9.3. Additional covariates that were used in sensitivity analyses included: breastfeeding, birthweight, offspring diabetes, hemoglobin A1c, and fasting glucose. See Figure 9.3 for directed acyclic graphs depicting the proposed relatedness between variables within each study design. Sensitivity analyses are described below.

Study designs and statistical analyses

Study 1 – Exact Matching. Matching is an important nonparametric approach to causal inference in observational research when treatment assignment is not under the investigator's control.[181] Matching uses strategic subsampling[181] to intuitively address causal questions and is unique in that it forces the investigator to confront the process of exposure and the limitations of the data.[115] For example, the individuals are matched on measured confounders, therefore to make any causal inferences you must assume any effect is not being driven by unmeasured confounding. Exact matching is the simplest version of matching and is what we applied in the present study. Although alternate matching methods exist (e.g., propensity score matching), exact matching is indicated when matching on fewer than eight covariates, and it reduces model dependence.[182]

After randomly selecting one mother-offspring pair per family, we had a cohort of 211,049 unique mother-offspring pairs. After removing all pregnancies complicated by type 2 diabetes and offspring with missing registration data (i.e., cases where person-years could not be calculated) we had a cohort of 209,417 mother-offspring pairs with gestational diabetes or with no diabetes available for matching. Using Godfrey et al., as a guide,[183] we matched all offspring exposed to gestational diabetes to one unexposed offspring, without replacement (i.e., an unexposed offspring could not be used in more than once match to an exposed offspring). We matched the unexposed offspring to the exposed offspring on urbanicity, RHA, SEFI-2, maternal age, parity, and offspring birth year. We performed exact matching on all categorial variables (i.e., urbanicity, RHA, and parity), but allowed continuous variables to vary to prioritize matching all exposed offspring and maximize external validity. We allowed maternal age and birth year to vary by 7 years and SEFI-2 by 0.7 points. These values were chosen because after several iterations, these were the lowest values we could allow the continuous variables to vary

and still match every exposed offspring. Due to the large sample, most exposed offspring had several unexposed matches. To select the closest match, we calculated a proximity score for each possible match using equation (1) below, where E = exposed and U = unexposed. The unexposed match with the lowest proximity score was selected for the one-to-one match of the exposed offspring. We assessed covariate balance between groups prior to analysis and found no significant differences in any of the continuous variables after matching (Table 5.2).

$$Proximity = \frac{[(Maternal\ Age_E - Maternal\ Age_U) + (SEFI2_E - SEFI2_U) + (Birth\ Year_E - Birth\ Year_U)]}{14.7} \quad (1)$$

After matching, we compared hypertension incident rates between exposed and unexposed offspring using a Poisson model with person-years of the denominator as an offset. All analyses were repeated on a cohort of mother-offspring pairs matched for exposure to type 2 diabetes during pregnancy. All data cleaning and analyses was completed using SAS software.

Sensitivity Analyses. We performed several sensitivity analyses beginning with adjustment for possible mediators of the association including breastfeeding, offspring birthweight, offspring size for gestational age at birth, and offspring diabetes (diagnosed before hypertension diagnosis). We also performed sex-stratified analyses and included offspring sex as an interaction term in the Poisson models to assess whether there was a sex-effect of exposure to diabetes in utero and offspring hypertension. To explore possible confounding by indication, we compared the prevalence of childhood physical examinations between the exposed and unexposed groups. A similar prevalence among exposed and unexposed offspring would suggest that neither group were more or less likely to encounter the healthcare system.

Study 2 - Paternal Negative Control. The use of negative controls is a method for detecting bias and confounding through selecting an exposure or outcome that is likely to involve the same bias

as the relationship of interest without involving the proposed causal mechanism.[184] Negative control analyses can apply negative exposure controls or negative outcomes. In the present study, we used paternal type 2 diabetes status at the time of offspring birth as a negative exposure control. The in-utero mechanism that is hypothesized to increase the offspring's incidence of hypertension is not present in the paternal negative control analysis; however, the confounding structure is likely similar. Therefore, a non-null result (i.e., a positive association between exposure to paternal diabetes prior to birth and offspring hypertension) would suggest that confounding (e.g., genetics or household environment) is biasing the result as, among paternal-offspring pairs, an intrauterine effect is impossible. This would challenge the causal nature of a positive association between exposure to intrauterine diabetes and incident offspring hypertension among maternal-offspring pairs given that the confounding factors are assumed to be similar among parents.

For this study, we began with the same cohort of mother-offspring pairs (n=418,169) and identified all offspring who were associated with a family male partner (n =259,303). The link between father and offspring was done through the Manitoba Health Registry, so it does not necessarily reflect a biological link between fathers and offspring. The father is the person registered as the family male partner (father) at the time of the child's birth. Sometimes there is no male partner at all, in which case father was listed as missing and those offspring were not included in the cohort. As with the maternal-offspring cohort, we removed fathers with type 1 diabetes, those with missing data on confounding variables and randomly selected one father-offspring pair from each family creating a cohort of 145,298 unique father-offspring pairs that were available for matching. We performed matching on the father-offspring pairs using the same method described above and completed the Poisson analyses as described above.

Sensitivity Analyses. We performed the same adjustments for potential mediating variables that were done in the maternal-offspring analysis. We also repeated the sex-stratified analyses and sex-interaction Poisson model.

Study 3 - Within Family Design: Discordant Sibling Analyses. A sibling analysis is a type of matched cohort study where the exposed individuals are matched to unexposed siblings as a way of adjusting for unmeasured shared environmental and genetic factors that are rarely available through administrative data. A null finding among siblings would imply that the in-utero exposure is not a causal mechanism, rather that the crude association is likely driven by shared familial confounding. Twin and sibling studies are popular matching designs because some, or all, confounding from genetics and shared familial characteristics are less likely to explain the observed associations.[185] In the present study we are limited to conducting a sibling analysis rather than a twin study as twins cannot be discordant for exposures in utero. However, the strength of comparing siblings rather than twins is that the intrauterine experience is more generalizable (i.e., the intrauterine environment of twins is different than singleton births). Discordant sibling studies have been used previously to assess intrauterine exposures research[186-190] but never to study the effect of diabetes on offspring hypertension, to our knowledge.

This sibling-matched study is a natural extension of the previous covariate-matched cohort design as it, at least partially, accounts for the unmeasured confounding that cannot be adjusted for, namely (partial) shared genetic risk and lifestyle factors. The key strength of this analysis is that it also controls for potentially important unmeasured familial confounding like the home environment.

We began with the same cohort of mother-offspring pairs (n=418,169) and identified all pregnancies that were complicated by gestational diabetes using the same method described

above. The offspring-pairs were included in the cohort if the mother had one child that was exposed to diabetes and at least one other child that was not exposed to any diabetes during pregnancy. If there were multiple non-exposed siblings to choose from, the sibling used in the analysis was selected at random to avoid introducing any selection bias. This left us with a cohort of 12,204 discordant sibling pairs. All mothers with type 1 diabetes were removed prior to matching. The primary analysis included all sibling pairs and was not adjusted for any additional potential confounders. We compared hypertension incidence among discordant siblings using conditional Poisson regression. The analysis was then repeated using siblings discordant for exposure to type 2 diabetes during pregnancy.

Sensitivity Analyses. Similar to the previous two analyses, we conducted sex-stratified analyses and performed a model with a sex-interaction. Additionally, misclassification bias is more likely among discordant sibling pairs than the general population because the pairs, by definition, differ on nonshared causes of the exposure and random measurement error is one such nonshared cause.[191] This means that there are more likely to be concordant sibling pairs misclassified as discordant which could bias our findings towards the null. To explore the extend to which this could bias our results, we compared measures of blood glucose including 1-hour, 2-hour, and fasting glucose and hemoglobin A1c in a small subset of the cohort where these data were available. This provided a crude estimate of differences in exposure to hyperglycemia between the sibling pairs.

Results

Study Cohort Characteristics

The characteristics of each study cohort used in analyses are shown in Table 5.1. Mean age across all parent-offspring pairs ranged from 27 to 37 years old, was highest among paternal-offspring pairs, and consistently increased after matching. SEFI-2 was also higher among all matched cohorts compared to all maternal- and paternal-offspring pairs, indicating more unfavourable socioeconomic conditions after matching. The paternal cohort had lower mean SEFI-2 overall compared to the maternal cohort (-0.13 and 0.04, respectively), indicating more favourable socioeconomic conditions among paternal-offspring pairs, compared to maternal-offspring pairs. The proportion of male and female offspring remained consistent across all three cohorts. The majority (44-57%) of parent-offspring pairs in all three cohorts resided in the Winnipeg Regional Health Authority; however, the proportion from the Northern Health Region doubled after matching in the maternal and paternal cohorts and nearly tripled after matching on discordant sib-ship. The prevalence of youth-onset hypertension was 3.0% among all mother-offspring pairs and 2.9% among all father-offspring pairs of unrelated siblings. Hypertension prevalence increased in all matched cohorts but was highest in the discordant sibling cohort (3.8%).

Table 5.1 Characteristics of each study cohort

	Cohort				
	Maternal-offspring pairs			Paternal-offspring pairs	
	Full cohort (unrelated siblings) n=209,417	Exact-matched n=11,612	Discordant siblings n=12,204	Full cohort (unrelated siblings) n=145,298	Exact-matched n=2,022
Parental characteristics at offspring's birth					
Age (years)	27 ± 6	29 ± 6	27 ± 6	31 ± 6	37 ± 8
SEFI-2	0.04 ± 1.05	0.37 ± 1.17	0.60 ± 1.24	-0.13 ± 0.95	0.25 ± 1.14
Regional Health Authority (%)					
Southern	13.3	7.4	8.1	15.7	10.1
Interlake-Eastern	8.2	8.6	10.9	8.2	8.8
Prairie Mountain	13.0	13.2	12.5	13.6	10.9
Northern	8.3	18.4	24.1	6.5	12.9
Winnipeg	57.1	52.3	44.4	55.9	57.3
Urbanicity (%)					
Urban	61.6	58.1	48.4	60.1	66.8
Rural	38.4	41.9	51.6	39.9	33.2
Offspring Characteristics					
Hypertension (%)	3.0	3.5	3.8	2.9	3.7
Age at diagnosis (years)	14 ± 5	13 ± 5	14 ± 5	14 ± 5	13 ± 5
Birthweight (g)	3512 ± 486	3585 ± 524	3691 ± 539	3519 ± 482	3527 ± 526
SGA (%)	9.0	7.28	5.2	8.7	8.1
LGA (%)	11.9	19.6	26.7	11.8	12.8
Biological Sex					
Male	51.0	51.9	51.3	51.0	50.0
Female	49.0	48.1	48.7	49.0	50.0

The maternal- and paternal-offspring pairs cohorts refer to all unrelated parent-offspring pairs prior to matching. The subsequent three matched cohorts were used for the primary analyses. The exact-matched cohort refers to maternal-offspring pairs after matching on potential confounding variables and the negative control cohort represents all paternal-offspring pairs after matching. SEFI-2, socioeconomic index factor version 2; SGA, small for gestational age; LGA, large for gestational age.

Exact Matching

Characteristics of mother-offspring pairs by exposure status in matched and unmatched cohorts can be found in Table 5.2. All exposed offspring from the mother-offspring cohort were

matched 1:1 to an unrelated offspring who was not exposed to gestational diabetes. All variables identified as potential confounders were balanced between exposed and unexposed offspring after matching. The proportion of males and females remained consistent after matching and was not different between exposed and unexposed offspring. Birthweight and the proportion of large for gestational age infants was significantly greater among offspring exposed to gestational diabetes. The prevalence of hypertension was significantly higher among exposed offspring compared to unexposed before and after matching (4.1 vs 3.0% and 4.1 vs 2.9%, respectively).

Table 5.2 Characteristics of maternal-offspring pairs by exposure to gestational diabetes in unmatched and matched sample.

	Unmatched Cohort (n = 209,417)			**Matched Cohort (n = 11,612)**		
	Exposed n = 5,806	**Unexposed** n = 203,611	**p-value**	**Exposed** n = 5,806	**Unexposed** n = 5,806	**p-value**
Maternal characteristics at offspring's birth						
Age (years)	29 ± 6	27 ± 6	<0.001	29 ± 6	29 ± 6	0.78
Parity (%)						
0	40.0	45.3	<0.001	40.0	40.0	1.0
1	30.4	33.7		30.4	30.4	
2	15.3	13.7		15.3	15.3	
3+	14.2	7.3		14.2	14.2	
SEFI – 2	0.38 ± 1.18	0.03 ± 1.05	<0.001	0.38 ± 1.18	0.36 ± 1.16	0.46
Regional Health Authority (%)						
Southern	7.4	13.5	<0.001	7.4	7.4	1.0
Interlake-Eastern	8.6	8.2		8.6	8.6	
Prairie Mountain	13.2	13.0		13.2	13.2	
Northern	18.4	8.1		18.4	18.4	
Winnipeg	52.3	57.2		52.3	52.3	
Urbanicity						
Urban	58.1	61.7	<0.001	58.1	58.1	1.0
Rural	41.9	38.3		41.9	41.9	
Offspring characteristics						
Birth year	1996 ± 9	1995 ± 9	<0.001	1996 ± 9	1996 ± 9	0.97
Hypertension (%)	4.1	3.0	<0.001	4.1	2.9	<0.001
Age at diagnosis	13 ± 5	14 ± 5	0.35	13 ± 5	12 ± 5	0.12
Birthweight (g)	3664 ± 549	3508 ± 483	<0.001	3664 ± 549	3507 ± 485	<0.001
SGA (%)	5.3	9.1	<0.001	5.3	9.2	<0.001
LGA (%)	27.5	11.4		27.5	11.8	
Biological Sex (%)						
Male	52.3	50.9	0.04	52.3	51.6	0.45
Female	47.7	49.1		47.7	48.4	

Values are mean ± standard deviation or percentage (%). LGA: large for gestational age; SGA: small for gestational age; urban: Winnipeg or Brandon; SEFI-2, socioeconomic index factor version 2.

Incident youth-onset hypertension among all unrelated offspring was 2.03/1,000 person-years. Rate of youth-onset hypertension was 2.72/1000 person-years among all offspring exposed to gestational diabetes and 2.01/1000 person-years among all unexposed offspring (IRR: 1.35; 95%CI: 1.19, 1.54). After matching, the rate of hypertension was 2.72 and 1.93/1,000 person-years among offspring exposed and unexposed to gestational diabetes, respectively

(IRR:1.41, 95%CI: 1.16,1.72). Before matching, the incidence of hypertension doubled (IRR: 2.14, 95%CI: 1.76, 2.62) between offspring exposed to type 2 diabetes during pregnancy (4.32/1,000 person-years) compared to unexposed offspring (2.01/1,000 person-years). After matching on potential confounding variables, the rate of hypertension among unexposed offspring was 1.71/1,000 person-years but remained higher among offspring exposed to type 2 diabetes (IRR: 2.53, 95%CI: 1.74, 3.68). Results from these analyses are shown in Figure 5.2 below. There were no sex-interaction effects in any of the models (data not shown).

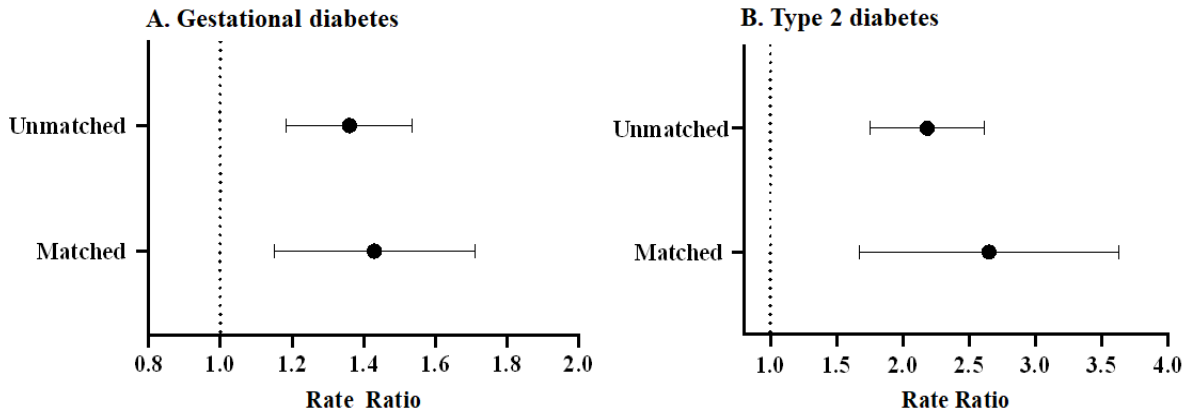

Figure 5.2 Offspring hypertension incidence rate ratio before and after matching among offspring exposed to gestational diabetes in utero (A) and maternal type 2 diabetes in utero (B).

Paternal Negative Control

Participant characteristics of the paternal negative control cohort before and after matching on confounding variables are described in Table 5.3. Two exposed offspring were not able to be matched so were removed from the matched cohort. However, removing these had negligible effects on the characteristics of the exposure group in the matched sample compared to the total cohort. The prevalence of type 2 diabetes among fathers was 0.7% which is similar to the prevalence of type 2 diabetes in the maternal cohort (0.8%), though it is considerably lower

than the prevalence of gestational diabetes (2.8%). Prior to matching, the age of diagnosis was lower among exposed compared to unexposed offspring, whereas after matching offspring age at diagnosis was similar (Table 5.3).

Table 5.3 Characteristics of offspring by exposure to paternal type 2 diabetes in full sample of father-offspring pairs and matched sample.

	Total Cohort (n = 145,298)			**Matched Cohort (n = 2,022)**		
	Exposed n = 1,013	**Unexposed** n = 144,285	**p-value**	**Exposed** n = 1,011	**Unexposed** n = 1,011	**p-value**
Paternal characteristics at offspring's birth						
Age (years)	37 ± 8	31 ± 6	<0.001	37 ± 8	37 ±8	0.88
SEFI – 2	0.25 ± 1.15	-0.13 ± 0.95	<0.001	0.25 ±1.15	0.25 ± 1.13	0.91
Regional Health Authority (%)						
Southern	10.2	15.8		10.1	10.1	
Interlake-Eastern	8.9	8.2		8.9	8.9	
Prairie Mountain	11.0	13.6	<0.001	11.0	11.0	1.0
Northern	12.9	6.5		12.9	12.9	
Winnipeg	57.1	55.9		57.2	57.2	
Urbanicity						
Urban	60.4	60.1	0.86	60.5	60.5	1.0
Rural	39.6	39.9		39.5	39.5	
Offspring characteristics						
Birth year	1998 ± 9	1992 ± 9	<0.001	1998 ± 9	1998 ± 9	0.93
Hypertension (%)	4.7	2.9	<0.001	4.7	3.2	0.07
Age at diagnosis (years)	12 ± 5	14 ± 5	0.004	12 ± 5	13 ± 5	0.32
Birthweight (g)	3520 ± 495	3519 ±482	0.99	3519 ± 496	3534 ± 513	0.58
SGA (%)	10.1	8.7	0.08	10.1	7.0	0.05
LGA (%)	13.3	11.8		13.2	13.6	
Biological Sex (%)						
Male	51.5	51.0	0.72	51.4	48.8	0.24
Female	48.5	49.0		48.6	51.2	

Values are mean ± standard deviation or percentage (%). LGA: large for gestational age; SGA: small for gestational age; urban: Winnipeg or Brandon; SEFI-2, socioeconomic index factor version 2.

The prevalence of hypertension was nearly 2-fold higher among offspring whose fathers had type 2 diabetes, compared to offspring whose fathers did not have diabetes (Table 5.3). This effect was similar before and after matching (Table 5.3). Among all paternal-offspring pairs

(n=145,298), hypertension incident rate was 3.26 and 1.92/1,000 person-years among offspring whose fathers did and did not have type 2 diabetes, respectively (IRR:1.70, 95% CI: 1.28, 2.27).

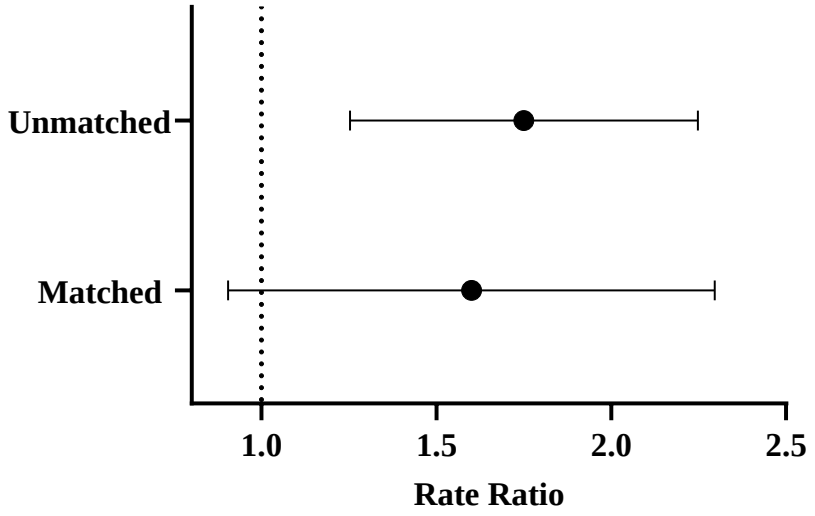

Figure 5.3 Offspring hypertension incidence rate ratio before and after matching among offspring whose fathers had type 2 diabetes at time of birth.

The sample was considerably smaller after matching (n=2,022) and hypertension rate among unexposed offspring increased slightly to 2.19/1,000 person-years. However, the effect size remained similar, though it was less precise (IRR:1.50, 95% CI: 0.96, 2.24) (Figure 5.2). There were no sex-interaction effects in any of the models (data not shown).

Within Family Design – Discordant Siblings

Out of a total of 383,660 mother-offspring pairs (inclusive of all pregnancies for each mother) in the entire cohort, 12,204 sibling pairs were discordant for exposure to gestational diabetes. Of note, the entire cohort of all mother-offspring pairs did not differ significantly from the cohort of mother-offspring pairs of unrelated offspring (Table 5.1) on any of the covariates (data not shown). After matching on sibship, there were no significant differences in SEFI-2,

regional health authority, or urbanicity between exposed and unexposed siblings (Table 5.4). Among discordant siblings, mothers of the exposed group were older and had higher parity compared to the unexposed group. Siblings exposed to gestational diabetes had higher birthweight and were more often born large for gestational age compared to unexposed siblings. Prior to matching the prevalence of youth-onset hypertension was 1.4 times higher among exposed compared to unexposed offspring and after matching there was no difference (Table 5.4).

The rate of youth-onset hypertension among siblings that exposed to gestational diabetes was 2.35/1,000 person-years and 2.31/1,000 person-years among siblings not exposed to diabetes (RR:1.01, 95%CI: 0.83,1.21). Similarly, the rate of hypertension among siblings exposed and unexposed to type 2 diabetes during gestation were 3.89 and 3.49/1,000 person-years, respectively (n = 3,308; RR: 0.98, 95%CI: 0.73, 1.31). Results are displayed in Figure 5.3. There were no sex-interaction effects in any of the models (data not shown).

Table 5.4 Characteristics of offspring by exposure to gestational diabetes in full sample of related mother-offspring pairs and discordant siblings.

	Total Cohort (n = 383,660)			**Matched Cohort (n = 12,204)**		
	Exposed n = 10,766	**Unexposed** n = 372,894	**p-value**	**Exposed** n = 6,102	**Unexposed** n = 6,102	**p-value**
Maternal characteristics at offspring's birth						
Age (years)	29 ± 6	27 ± 6	<0.001	28 ± 6	26 ± 6	<0.001
Parity (%)						
0	31.6	39.1	<0.001	23.0	35.6	<0.001
1	29.3	33.9		31.3	33.1	
2	17.6	16.1		20.2	15.5	
3+	21.5	10.9		25.6	15.8	
SEFI – 2	0.56 ± 1.24	0.13 ± 1.09	<0.001	0.61 ± 1.26	0.60 ± 1.23	0.70
Regional Health Authority (%)						
Southern	7.3	14.9	<0.001	8.0	8.1	0.95
Interlake-Eastern	9.6	8.7		10.7	11.1	
Prairie Mountain	13.2	13.2		12.7	12.4	
Northern	22.8	9.8		24.2	24.0	
Winnipeg	47.0	53.3		44.4	44.4	
Urbanicity						
Urban	51.9	57.4	<0.001	48.7	48.1	0.95
Rural	48.1	42.6		51.3	51.9	
Offspring characteristics						
Birth year	1996 ± 8	1995 ± 9	<0.001	1996 ± 8	1994 ± 9	<0.001
Hypertension (%)	4.2	3.0	<0.001	3.8	3.8	0.96
Age at diagnosis	13 ± 5	14 ± 5	0.003	13 ± 5	14 ± 5	0.14
Birthweight (g)	3690 ± 549	3525 ± 483	<0.001	3710 ± 546	3673 ± 531	<0.001
SGA (%)	5.0	8.6	<0.001	4.9	5.6	<0.001
LGA (%)	29.2	12.1		29.3	24.1	
Biological Sex (%)						
Male	52.4	50.9	0.001	52.8	49.9	0.001
Female	47.6	49.1		47.2	50.1	

Values are mean ± standard deviation or percentage (%). LGA: large for gestational age; SGA: small for gestational age; urban: Winnipeg or Brandon; SEFI-2, socioeconomic index factor version 2.

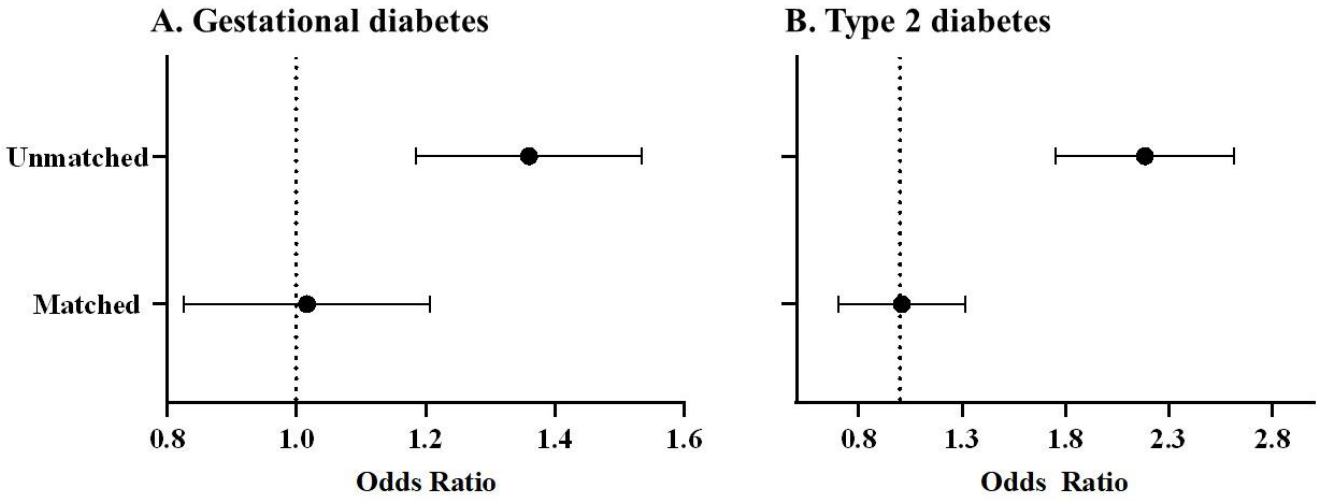

Figure 5.4 Offspring hypertension incidence before and after matching among siblings discordant for exposure to gestational diabetes (A) and maternal type 2 diabetes in utero (B).

Discussion

Triangulating the results of several analyses completed within a large administrative dataset suggest that the association between gestational diabetes and offspring hypertension observed here and in previous observational studies is likely driven, to a large extent, by unmeasured confounding rather than a direct in-utero mechanism. The rate of hypertension among offspring exposed to gestational diabetes was ~1.4 times higher than unexposed offspring matched for observed confounding. However, in the negative control matched analysis – using father-offspring pairs and paternal type 2 diabetes as the negative exposure – there was a similar effect size, indicating that the association observed between exposure to gestational diabetes and offspring hypertension among maternal-offspring pairs was likely biased by shared unmeasured confounding. Lastly, there was no difference in the incidence of youth-onset hypertension among sibling pairs that were discordant for exposure to gestational diabetes in utero. Taken together, if an in-utero mechanism were driving the association that we observed among the between-family

matched mother-offspring pairs, then we would expect (1) a null result in the paternal negative control analysis and (2) a positive effect in the discordant sibling comparison. In contrast, we observed the opposite suggesting that confounding, rather than an in-utero mechanism, was a major driver of the observed association.

Several previous observational studies have demonstrated that exposure to diabetes in utero is associated with an elevated risk of hypertension and high blood pressure among offspring; however, there are some discrepancies within the literature. Similar to others,[173, 192] the results from study 1 (the covariate matched cohort) showed a 40% higher rate of youth-onset hypertension in offspring exposed to maternal gestational diabetes (among unrelated offspring) matched for measured confounders. Also, in keeping with previous literature,[193] we saw a greater effect among offspring exposed to type 2 diabetes (Figure 5.2), suggesting a potential dose-response. Others have reported elevated systolic blood pressure among offspring who were exposed to gestational diabetes during pregnancy compared to unexposed offspring,[72, 194] while another study reported elevated systolic blood pressure without a concomitant increase in the prevalence of hypertension between offspring exposed and unexposed to gestational diabetes.[12] Multiple studies cite altered vascular function[195] and altered organ development[86, 196] as primary mechanisms driving the observed increase in blood pressure among offspring exposed to diabetes in utero. However, evidence of these mechanisms in humans is lacking,[98] and interventions aimed at treating gestational diabetes have not translated into more favourable cardiovascular specific outcomes for offspring.[197, 198] This lack of mechanistic evidence in humans and the limited effectiveness of interventions align with the results from our negative control and within-siblings analyses, which did not support the role of a causal in-utero

mechanism driving the association between exposure to diabetes in utero and offspring hypertension incidence.

Confounding bias is arguably one of the greatest challenges to conducting causal investigations within observational datasets. The present investigation revealed that unmeasured confounding is most likely driving the discrepancy between the findings from the between-family matched mother-offspring pairs analysis and the paternal negative control and sibling analyses. Among father-offspring pairs we saw similar trends in measured confounding between exposed and unexposed offspring as we did in mother-offspring pairs including higher socioeconomic deprivation, higher parental age, and a greater proportion living in northern rural and remote areas (Tables 5.2 and 5.3). This supports the assumption that unmeasured confounding, such as neighbourhood factors, structural factors and individual lifestyle behaviours like diet and exercise, are likely similar between father- and mother-offspring pairs. Collectively, these data suggest that unmeasured confounding, not an in-utero mechanism, likely explains the increased rate of hypertension among exposed offspring in the father-offspring cohort and mother-offspring cohort (Figure 5.1 and 5.2). Similarly, between discordant siblings – without additional adjustment – measured confounding variables like socioeconomic deprivation and living in a rural or remote area were not different between exposed and unexposed siblings (Table 5.4). These data support the assumption that discordant siblings are exposed to similar home environments and thus comparing discordant siblings inherently adjusts for these, and likely other unmeasured, confounding. The negligible difference in hypertension rate among siblings discordant for exposure to both gestational and type 2 diabetes provides additional evidence that extrauterine factors were likely driving the association seen in the measured confounding matched analysis.

Limitations and Sensitivity Analyses

Importantly, there are some limitations to consider. It is possible that fathers with type 2 diabetes are more likely to have partners who also have diabetes, therefore offspring exposed to fathers with type 2 diabetes may also be differentially exposed to diabetes in utero which could also be driving the observed effect. However, the effect estimates did not change when the paternal negative control models were adjusted for maternal diabetes status (Table 9.7). Additionally, there may be a higher risk of misclassification of the exposure in the sibling study which could bias the effect towards the null. However, we observed higher mean fasting and 2-hour blood glucose among a subset of mothers (1.3%) in the sibling cohort who were categorized as having gestational diabetes compared to those who weren't (Table 9.8). I was also concerned about confounding by indication in identifying youth-onset hypertension.[199]After comparing prevalence of childhood physicals, which occurred prior to offspring hypertension diagnosis, there was no difference in the proportion of child physicals among offspring with and without hypertension diagnoses (Table 9.9).[199]After comparing prevalence of childhood physicals, which occurred prior to offspring hypertension diagnosis, there was no difference in the proportion of child physicals among offspring with and without hypertension diagnoses (Table 9.9). Lastly, it is important to consider that, although it has been validated, the administrative health data-based algorithm used here to diagnose youth-onset hypertension has modest sensitivity,[59] suggesting that hypertension is likely underestimated. However, it is unlikely that this would systematically affect one exposure group more than the other and significantly bias the results presented here.

Conclusions

The triangulation of data presented here suggest that confounding bias was likely a major driver in the association between exposure to diabetes during pregnancy and increased rate of hypertension in offspring and may be a factor driving several of the positive associations reported in the literature.

Acknowledgements

The authors acknowledge the Manitoba Centre for Health Policy for use of data contained in the Manitoba Population Research Data Repository. Data used in this study are from the Manitoba Population Research Data Repository housed at the Manitoba Centre for Health Policy, University of Manitoba and were derived from data provided by Manitoba Health and the Winnipeg Regional Health Authority. The final draft of the manuscript was sent to the data providers (the Health Information Privacy Committee [HIPC] and the Winnipeg Regional Health Authority) regarding confidentiality, privacy and consistency with the HIPC-approved protocol, but they had no control on the results presented or the decision to submit the manuscript for publication. We heartfully thank Mr. Randy Walld for his logistical support in cutting the required data.

Chapter 6: Extended Discussion

The prevalence of hypertension is increasing among children and adolescents,[68] and with that, there is a concomitant need to delineate causal pathways. The decreasing age at which hypertension is occurring has sparked continued interest in the role of early life, particularly intrauterine, exposures that could predispose individuals to developing this chronic condition. Given the previous work linking the intrauterine environment with subsequent cardiometabolic conditions,[7, 24, 35, 72, 78, 90, 145, 174, 178, 186, 200] including blood pressure,[11, 12, 67, 75, 83, 96, 194] combined with the rising prevalence of metabolic disturbances in pregnancy,[8, 121, 160] this has been an area of focus for scientists trying to elucidate the natural history of hypertension, particularly in youth.

In Chapter 2, I provided a review of the literature describing in-utero exposure to diabetes and subsequent risk of high blood pressure or hypertension in offspring, highlighting the limitations and discrepancies within the data. In particular, several seminal studies used causal language to describe their findings without explicitly discussing causal inference.[7, 79, 201] More recent work, with causal inference at the forefront of the investigation, has brought previously held assumptions about intrauterine origins of disease into question.[102, 202, 203] Therefore, the current dissertation was guided by the primary research questions: (1) has the incidence of gestational diabetes changed over the last several decades among high risk groups? and (2) does the well-established relationship between exposure to gestational diabetes during pregnancy and development of offspring hypertension reflect a causal in-utero mechanism?

To provide context for the primary exposure of interest, gestational diabetes, I completed the descriptive study presented in Chapter 4. To investigate the primary causal question,

acknowledging the limitations of observational data, I completed the triangulation study presented in Chapter 5. Together these investigations provided the necessary data to understand the state of gestational diabetes in the province and the potential implications. Inspired by the theoretical framework underlying this dissertation (Figure 3.1), the remaining sections will: reiterate the key findings from the studies, further discuss some of the nuance of the data and other considerations, describe how the results from this thesis contribute to causal inference-based studies within the life course literature on exposure to diabetes in utero and offspring hypertension, and review key limitations and proposed future directions.

6.1 Overview of findings

In Chapter 4 I demonstrated that gestational diabetes has increased dramatically in Manitoba since 1981, in concordance with other provinces in Canada,[123-125, 160] and internationally.[121, 127, 161] The increase was characterized by an upward inflection that is likely related to the publication of the International Association of the Diabetes and Pregnancy Study Groups recommendation calling for more sensitive screening practices.[204] Uptake of more sensitive screening practices means that individuals with less severe deviations from normal will be categorized as having gestational diabetes. Therefore, it is difficult to conclude whether the higher incidence of gestational diabetes in recent years is reflective of worsening health or is indicative of a more proactive and preventative approach to healthcare which could improve the outcomes of these folks and their offspring. Importantly, we saw a relatively greater increase among those above age 35, living in urban areas, and living in the Northern Health Authority, suggesting that the increase in diagnoses is not equally distributed among all pregnant Manitobans. The disparities in the relative increased rates of gestational diabetes in older pregnant persons, those in the highest SES and higher resourced areas suggest that changes in

diagnostic criteria certainly play a role in the rising rates, however the persistently higher rates in northern regions and among the lowest SES suggest that some sub-groups within the larger population are experiencing worsening pregnancy health outcomes.

The triangulation of three unique studies each with different sources of bias presented in Chapter 5 did not provide evidence in support of a direct in-utero mechanism for the development of hypertension in offspring. Despite a positive effect in the maternal-offspring covariate-matched sample, results from the paternal negative exposure control and sibling studies did not support a causal in-utero mechanism. The use of multiple study designs was imperative here given the methodological challenges, described in Chapter 2, in studying intrauterine exposures and distal offspring health outcomes like hypertension. Particularly, the potential bias from residual confounding that is often not captured in administrative databases. The different and complimentary management of confounding within the three distinct methods provide robust evidence to conclude that the increased rate of hypertension seen among offspring exposed to gestational diabetes in utero was likely driven by confounding bias. Still, there are methodological nuances within these studies that are critical to consider.

6.2 Additional considerations for causal inferences

The purpose of the triangulation investigation presented in Chapter 5 was to draw causal inferences about the relationship between exposure to gestational diabetes in utero and incident youth-onset hypertension in offspring. Our results demonstrated that a direct causal effect is unlikely. Though this conclusion is supported by the composite results from the three separate studies, there are several important assumptions and methodological caveats to carefully consider. Namely, the specific causal estimate investigated and associated assumptions, the

potential for misclassification, and the complexity of the proposed causal pathway – one that is likely comprised of several interacting confounders, mediators, and modifiers (Figure 9.3).

It is important to be explicit about the causal estimate that was investigated in Chapter 5. First, the causal estimate is largely based on estimating the counterfactual and evidence of regularity (Table 2.1). By applying various matching techniques, the aim was to compare the exposed offspring to a group of similar unexposed offspring to estimate what would have happened to those offspring had they not been exposed (i.e., estimate the counterfactual). I also relied on regularity, or lack thereof, in the negative control study. I would expect to regularly see an association between the exposure (gestational diabetes) and the outcome (offspring hypertension) if a causal in-utero mechanism is driving the association. However, replacing the in-utero exposure with a similar exposure (i.e., paternal type 2 diabetes) that does not confer an intrauterine effect (i.e., the exposure is "negative") means that I would not expect to see the regularity with the negative exposure that would otherwise be observed with the causal exposure. Additionally, in these analyses, I estimated the average treatment effect of the treated, meaning that I made inferences about the exposed group had they been unexposed and can not make inferences about what would have happened to the unexposed offspring had they been exposed. This is because of the relatively large proportion of unexposed offspring (~98% of cohort) compared to exposed offspring. Therefore, it would not have been feasible to match all unexposed offspring and instead I matched every exposed offspring. The benefit of a rare exposure being that I was able to match every single exposed offspring, however, I was not able to match a large proportion of the unexposed offspring. That means, the data do not support inferences about how a change in exposure status might have affected the unexposed group.

There are three key assumptions to consider when estimating a causal effect: exchangeability, positivity, and consistency.[205] Exchangeability refers to there being no unmeasured confounding so that the treatment assignment mechanism (i.e., the factors that lead to an individual being exposed or treated) is ignorable. Exchangeability also incorporates the assumption that there is no informative censoring, between exposed and unexposed individuals.[205] If the exchangeability assumption is satisfied, then the exposed and unexposed groups are said to be "exchangeable". In other words, exchangeability means that if you were to switch the exposure status of the exposed and unexposed groups, the outcome of the study would be exactly the same. The positivity assumption states that there is a positive, or non-zero, probability of receiving the exposure for every individual at every level of all confounders that occur in the population. The consistency assumption states that the exposure is clearly defined and the outcome under an individual's observed exposure would be the same had the individual been assigned that exposure.[205, 206]

For observational studies where causal inference is the objective, as is the case in the study presented in Chapter 5, it is important to consider the extent to which each of the assumptions described above are satisfied. The exchangeability assumption is challenging to satisfy when using observational data due to the inability to control and randomly assign the exposure; therefore, the risk of unmeasured confounding in these studies is high. However, triangulating the results of three different studies strengthens our ability to satisfy this assumption because each study treats confounding in a different way (Figure 5.1). The positivity assumption is largely met in this study because any offspring could feasibly be exposed to gestational diabetes regardless of the level of any of the observed confounders or conceivable unobserved confounders. The most challenging assumption to meet for the work described in

Chapter 5 is consistency. Consistency is particularly difficult to satisfy when using a biological exposure, like gestational diabetes, because there are several ways an individual could be hypothetically "assigned" to being exposed to gestational diabetes in utero. The threat to consistency is that there could be too much variation in the exposure, resulting in a different outcome if the specification of the exposure differed.[206] However, as described in Chapter 5, there was a similar increased risk for hypertension among offspring exposed to gestational diabetes as what has been reported in other studies.[11, 193] This lends support for assuming consistency because, despite slight differences in how gestational diabetes was specified in each study, the effects were similar, suggesting that a similar exposure was captured.

In addition to exchangeability, positivity, and consistency, misclassification bias is an important consideration, particularly when using administrative data where the data were not collected for the purpose of the specific research project. The exposure of gestational diabetes is at risk of misclassification bias because of the variability in diagnosing the disease, especially within administrative databases when relying on ICD coding and prescription data.[207] There may be differences in how and whether physicians apply updated guidelines which could lead to varying severities of metabolic dysregulation during pregnancy that is aggregated in our binary exposure. This bias is a particularly relevant to the analysis among discordant siblings because, as mentioned previously in Chapter 5, selecting on the fact that diabetes diagnosis in pregnancy resulted in different sibling exposures means that and misclassification is one of the reasons this could occur. However, in a small sub-sample where blood glucose data was available, we saw higher blood glucose among those with gestational diabetes (Table 9.8), which supports the validity of our exposure and supports our conclusion that an intrauterine exposure is an unlikely cause for developing youth-onset hypertension.

6.3 Contributions to developmental origins and life course epidemiology

Results from this dissertation support the theory of life course epidemiology accumulation of risk pathway rather than fetal "programming" (i.e., predictive or immediate adaptive responses) or critical windows theories described previously.[55] Based on the findings from this study, I argue that there is unlikely a clinically meaningful fetal developmental affect on the risk for youth-onset hypertension following exposure to gestational diabetes in utero. In other words, even if there is a subtle direct developmental effect on offspring exposed to gestational diabetes that increases their risk of developing hypertension, it is unlikely to alone have a meaningful impact on the offspring's long-term health. Therefore, a major implication from this work to the broader developmental origins of health and disease literature is to place more emphasis on extrauterine factors that contribute to both elevated incidence of hypertension among youth and gestational diabetes.

Within the developmental origins literature there is an over-emphasis on studying maternal and fetal exposures relative to work investigating paternal and extrauterine exposures.[208] This is reflective of long-held assumptions about maternal and intrauterine exposures having a greater impact on future health relative to paternal and postnatal factors. This assumption that the mother's health plays the most important role in the health of the offspring drove early developmental origins research and has since contributed to a looping effect where study designs, interpretations, and policies are all rooted in studying maternal health rather than considering other alternatives.[19] Structural factors like poverty and racism play critical roles in the etiology of both gestational diabetes[209] and pediatric hypertension.[210] Results from this thesis highlight the importance of studying extrauterine factors like these on developmental health given the lack of evidence of a causal relationship between maternal gestational diabetes and

offspring hypertension in youth described in Chapter 5. Therefore, we should be cautious about inadvertently dismissing structural drivers of disease in favour of focusing on individuals' behaviours because it can cause undue harm to mothers,[211] and drastically oversimplify the complex etiology of chronic disease. I think the work presented here supports a rationale for studying structural factors that may shape both development of gestational diabetes and youth-onset hypertension. Also, I think this work can help shift some of the high burden of responsibility that is placed on mothers by reinforcing the importance of extrauterine factors that influence offspring health and development, including paternal health. Dialogue between clinicians and patients should include discussions of extrauterine determinants of health to de-center maternal health as the primary causal factor in offspring growth and development.

The results from this thesis also demonstrate the need to fill the gap between tightly controlled mechanistic animal studies and human epidemiology studies where biomedical variables interact with behavioural and psychosocial variables. Well-designed animal models of obesity and diabetes have provided clear evidence of an intrauterine effect,[13] but this has not clearly translated into human disease. In the context of these animal studies our findings, again, emphasize the importance of considering the behavioural and psychosocial elements of chronic disease in humans. Additionally, the causal web becomes increasingly complex the more distal the outcome is from the exposure. It is important to capture a sample in which a chronic disease has had time to develop; however, the further in time the outcome is from the exposure, the greater the complexity with regards to modifiers and mediating variables that can become difficult to clarify.

Methodologically, the studies presented here provide an example of maximizing the wealth of data often available within administrative health databases. Through leveraging this

large, representative sample of parents, offspring, and siblings, I was able to describe trends in gestational diabetes incidence going back several decades and conduct a causal investigation of a developmental origins hypothesis commonly described in the literature. Through applying triangulation, and drawing on other relatively recently discussed methods and models for causal inference using observational data,[18, 20, 106, 111, 115, 181, 184] I was able to discuss causality in a context in which a randomized controlled trial would never be possible. For other scientists with access to large datasets that may lack granularity, this work provides a practical example of the value of the data and the breadth of possibilities. With careful consideration and application of emerging methods (e.g., Triangulation, Mendelian randomization, target trial emulation, G-computation, or inverse probability weighting) more robust causal investigations into early origin hypotheses can be accomplished.

The theoretical frameworks of causal inference and developmental origins of disease intersect in this work to explicitly study the causal effect of exposure to gestational diabetes in utero on the development of pediatric hypertension in the offspring using a triangulation approach. There are several instances within the developmental origins literature where causality is implied, though not explicitly discussed.[24, 174, 194, 196] This is problematic because it can lead to erroneous causal conclusions that are not specifically tested or assessed for satisfying assumptions. As others have done with varying early life exposures,[177, 187, 202] our work precisely defines our causal estimates in the context of the studies' various limitations, assumptions, and biases. Though the hesitation around discussing causation, given the methodological limitations surrounding this type of work, is understandable, it is not productive; it does not allow for frank discussion about the true objective and limitations of assessing causation when studying early life exposures and later offspring health outcomes. Much of the work within developmental

origins literature is inherently causal, and I think the work presented in this thesis adds to the literature by providing an example of a transparent approach to investigating a difficult causal question through the application of triangulation within an administrative dataset.

Work from this thesis informs the developmental origins and life course literature because it demonstrates the importance of considering unobserved confounders that might be driving the association. It also draws attention to the complexity of the relationship between an in-utero exposure and distal offspring outcome.

6.4 Limitations and Future Directions

As with all research, there are limitations that are important to consider. First, all analyses were done using the same data source. Despite it being an extremely rich source of data, the limitations described previously surrounding misclassification are consistent between all studies. Similarly, despite having data on the entire province of Manitoba, it is not a densely populated province, so it is possible we were underpowered to detect very small effects. Unlike a study recently done in Ontario where the sample size contained millions of mother-offspring pairs. Applying the methods described here to a similarly large dataset outside of Manitoba would provide a powerful follow-up to the present dissertation.

Additionally, this work is limited by our ability to accurately capture the true incidence of youth-onset hypertension. Previous work has demonstrated that pediatric hypertension is underestimated in administrative databases, where the sensitivity was only 0.51.[59] Therefore, it is likely that we were unable to identify all cases of hypertension within our dataset. This is problematic for two main reasons: (1) we have fewer endpoints to study and therefore less power, and (2) that one group may be more likely to experience underdiagnosis in the

administrative data, potentially biasing our result by confounding by indication. Given that we saw a statistically significant effect between offspring matched on potential covariates (Figure 5.2) the only analysis where power might have been an issue was in the paternal-offspring pair analysis. However, given that there was still an effect suggests that having more end points would not have likely changed our interpretation. To address potential bias by indication, we compared the proportion of well-child visits between groups and saw no difference (Table 9.8). It is possible that screening for pediatric hypertension, which has been shown to be low in Canada,[65] may also be systematically lower in one group however, blood pressure measurements have been shown to be more common among high risk youth (e.g., those with overweight or obesity),[65] which would bias the results towards an effect and that is the opposite of our conclusion, particularly considering the sibling analysis. Therefore, even with a perfectly accurate estimate of the incidence of pediatric hypertension, our results likely would not change. Though, future studies should prioritize how to accurately capture the true prevalence of pediatric hypertension.

Finally, these studies are limited by the lack of individual-level data for race and ethnicity and several potential confounding variables (e.g., income was defined at the neighbourhood level). This affected the study described in Chapter 4 because we were not able to determine if the trends in gestational diabetes incidence over time were different for structurally oppressed groups, and, in Chapter 5, there may be greater variability among some covariate-matched exposed and unexposed offspring given the potential variation within neighbourhood categories. However, it is unlikely that this limitation significantly biased our findings as there was a notably higher rate of hypertension among offspring in both covariate-matched analyses (Figure 5.2 and 5.3) and no effect in the sibling-analysis (Figure 5.4). Additionally, not being able to describe

sub-groups by individual-level covariates or by ethnicity in Chapter 4 does not invalidate the findings we presented, but rather points to an important area of future work. Both the descriptive study and triangulation study described in this thesis could be strengthened by additional individual-level confounding to extend on the present findings but would unlikely change our interpretations.

This work demonstrates the need to target specific exposures and assess proximal outcomes. We need more sensitive measures within the large cohort studies to fill the gap between the mechanistic animal studies and human epidemiology studies. Additionally, there is a need for further investigation into behavioural and psychosocial variables. Most importantly, scientists in this area should continue to apply evidential pluralism approaches like triangulation. Understanding a complex causal web like that of developing hypertension requires multiple lines of investigation and multiple sources of data. Given the data presented here and by others in recent years, it seems that intrauterine "programming" is likely, in most cases, to be an oversimplistic explanation for the development of complex chronic conditions like hypertension.

Chapter 7: Conclusion

The objectives of this dissertation were to describe the population trends in gestational diabetes incidence and investigate whether exposure to gestational diabetes in utero confers a direct effect on fetal development that increases their risk of developing hypertension throughout childhood and adolescence. I have accomplished both of these objectives as outlined in Chapter 4 and 5. Briefly, I have demonstrated that gestational diabetes is on the rise. However, the marked increase of cases over the last decade may not necessarily represent higher levels of glycemic exposure but rather more sensitive diagnostic criteria. I have also demonstrated that gestational diabetes is unlikely to be a direct independent risk factor for the development of hypertension in youth among exposed offspring. The evidence from the triangulation approach suggests that this association may be completely driven by confounding bias. Figure 7.1 provides an updated theoretical framework based on the present findings, which suggests that offspring exposed to gestational diabetes are more likely to develop hypertension due to other factors that are commonly associated with gestational diabetes rather than being born with an innate predisposition due to an intrauterine mechanism.

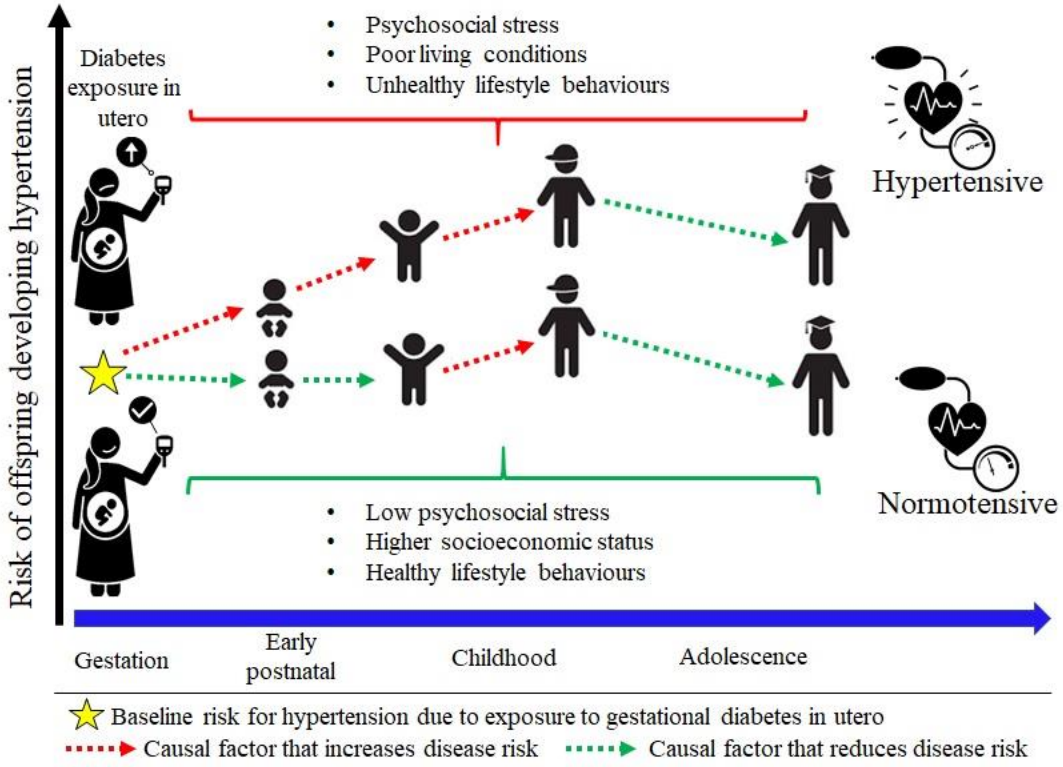

Figure 7.1 Updated Theoretical Framework

The work completed in this thesis demonstrates the need to explore other elements of the complex causal web that leads to the development of youth-onset hypertension. One such avenue of future research is to identify the primary confounder, or confounders, driving the association. By doing so, we can begin to focus on what may be not only contributing to elevated hypertension risk among offspring, but also increasing the risk of developing diabetes in pregnancy.

Chapter 8: References

1. Smith CA. Effect of wartime starvation in Holland upon pregnancy and infant health. Nutrition Reviews. 1947;5(9):276-7.
2. Antonov AN. Children born during the siege of Leningrad in 1942. J Pediatr. 1947;30(3):250-9.
3. Stein Z, Susser M. The Dutch famine, 1944-1945, and the reproductive process. I. Effects on six indices at birth. Pediatr Res. 1975;9(2):70-6.
4. Rudolski L. On pregnancy in animals in cases of insufficient nourishment of the organism; experimental investigations performed upon rabbits and a dog.(Russian.) Dissert. St Petersburg. 1893.
5. Painter RC, de Rooij SR, Bossuyt PM, de Groot E, Stok WJ, Osmond C, et al. Maternal nutrition during gestation and carotid arterial compliance in the adult offspring: the Dutch famine birth cohort. J Hypertens. 2007;25(3):533-40.
6. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev. 2006;82(8):485-91.
7. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ. 1989;298(6673):564-7.
8. Chiefari E, Arcidiacono B, Foti D, Brunetti A. Gestational diabetes mellitus: an updated overview. J Endocrinol Invest. 2017;40(9):899-909.
9. Bunt JC, Tataranni PA, Salbe AD. Intrauterine exposure to diabetes is a determinant of hemoglobin A(1)c and systolic blood pressure in pima Indian children. J Clin Endocrinol Metab. 2005;90(6):3225-9.
10. Kelly BA, Lewandowski AJ, Worton SA, Davis EF, Lazdam M, Francis J, et al. Antenatal glucocorticoid exposure and long-term alterations in aortic function and glucose metabolism. Pediatrics. 2012;129(5):e1282-90.
11. Lu J, Zhang S, Li W, Leng J, Wang L, Liu H, et al. Maternal Gestational Diabetes Is Associated With Offspring's Hypertension. Am J Hypertens. 2019;32(4):335-42.
12. Miranda JO, Cerqueira RJ, Barros H, Areias JC. Maternal Diabetes Mellitus as a Risk Factor for High Blood Pressure in Late Childhood. Hypertension (Dallas, Tex : 1979). 2019;73(1):e1-e7.
13. Agarwal P, Morriseau TS, Kereliuk SM, Doucette CA, Wicklow BA, Dolinsky VW. Maternal obesity, diabetes during pregnancy and epigenetic mechanisms that influence the developmental origins of cardiometabolic disease in the offspring. Crit Rev Clin Lab Sci. 2018;55(2):71-101.
14. Yan J, Li X, Su R, Zhang K, Yang H. Long-term effects of maternal diabetes on blood pressure and renal function in rat male offspring. PLoS One. 2014;9(2):e88269.
15. Yu C, Chen S, Wang X, Wu G, Zhang Y, Fu C, et al. Exposure to maternal diabetes induces endothelial dysfunction and hypertension in adult male rat offspring. Microvasc Res. 2021;133:104076.
16. Yuan WL, Lin J, Kramer MS, Godfrey KM, Gluckman PD, Chong YS, et al. Maternal Glycemia During Pregnancy and Child Carotid Intima Media Thickness, Pulse Wave Velocity, and Augmentation Index. J Clin Endocrinol Metab. 2020;105(7).

17. Amrithraj AI, Kodali A, Nguyen L, Teo AKK, Chang CW, Karnani N, et al. Gestational Diabetes Alters Functions in Offspring's Umbilical Cord Cells With Implications for Cardiovascular Health. Endocrinology. 2017;158(7):2102-12.
18. Krieger N, Davey Smith G. The tale wagged by the DAG: broadening the scope of causal inference and explanation for epidemiology. Int J Epidemiol. 2016;45(6):1787-808.
19. Sharp GC, Lawlor DA, Richardson SS. It's the mother!: How assumptions about the causal primacy of maternal effects influence research on the developmental origins of health and disease. Soc Sci Med. 2018;213:20-7.
20. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. Int J Epidemiol. 2016;45(6):1866-86.
21. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet (London, England). 1986;1(8489):1077-81.
22. Disease ISfDOoHa. Who We Are https://dohadsoc.org/who-we-are/2022 [Available from: https://dohadsoc.org/who-we-are/.
23. Poore KR, Hanson MA, Faustman EM, Neira M. Avoidable early life environmental exposures. Lancet Planet Health. 2017;1(5):e172-e3.
24. Barker DJ. Maternal nutrition and cardiovascular disease. Nutr Health. 1993;9(2):99-106.
25. Neel JV. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? American journal of human genetics. 1962;14(4):353.
26. Gluckman PD, Hanson MA. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. Pediatr Res. 2004;56(3):311-7.
27. Rosenfeld CS. The Epigenome and Developmental Origins of Health and Disease. London: Academic Press; 2016.
28. Neel JV. The “thrifty genotype” in 1998 1. 1999.
29. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35(7):595-601.
30. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61-73.
31. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5-20.
32. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? Physiological reviews. 2014;94(4):1027-76.
33. Gillman MW, Barker D, Bier D, Cagampang F, Challis J, Fall C, et al. Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). Pediatr Res. 2007;61(5 Pt 1):625-9.
34. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. Science. 2004;305(5691):1733-6.
35. Luyckx VA, Brenner BM. Birth weight, malnutrition and kidney-associated outcomes—a global concern. Nature Reviews Nephrology. 2015;11(3):135-49.
36. Mesquita F, Gontijo J, Boer P. Maternal undernutrition and the offspring kidney: from fetal to adult life. Brazilian Journal of Medical and Biological Research. 2010;43:1010-8.
37. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med. 1976;295(7):349-53.
38. Imdad A, Lassi Z, Salaam R, Bhutta ZA. Prenatal nutrition and nutrition in pregnancy: effects on long-term growth and development. Early Nutrition and Long-Term Health: Elsevier; 2017. p. 3-24.

39. Disease ISfDOoHa. The Cape Town Manifesto. International Society for Developmental Origins of Health and Disease, Cape Town2015.
40. Russo VE, Martienssen RA, Riggs AD. Epigenetic mechanisms of gene regulation1996.
41. Deodati A, Inzaghi E, Cianfarani S. Epigenetics and in utero acquired predisposition to metabolic disease. Front Genet 10: 1270. 2019.
42. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature Genetics. 2003;33:245+.
43. Safi-Stibler S, Gabory A, editors. Epigenetics and the Developmental Origins of Health and Disease: Parental environment signalling to the epigenome, critical time windows and sculpting the adult phenotype. Seminars in cell & developmental biology; 2020: Elsevier.
44. Dolinoy DC, Das R, Weidman JR, Jirtle RL. Metastable epialleles, imprinting, and the fetal origins of adult diseases. Pediatric research. 2007;61(7):30-7.
45. Simeoni U, Barker DJ, editors. Offspring of diabetic pregnancy: long-term outcomes. Seminars in Fetal and Neonatal Medicine; 2009: Elsevier.
46. Toraño EG, García MG, Fernández-Morera JL, Niño-García P, Fernández AF. The impact of external factors on the epigenome: in utero and over lifetime. BioMed research international. 2016;2016(1):2568635.
47. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proceedings of the National Academy of Sciences. 2008;105(44):17046-9.
48. Tobi EW, Goeman JJ, Monajemi R, Gu H, Putter H, Zhang Y, et al. DNA methylation signatures link prenatal famine exposure to growth and metabolism. Nature communications. 2014;5(1):5592.
49. Vaiserman A, Lushchak O. Prenatal famine exposure and adult health outcomes: an epigenetic link. Environmental Epigenetics. 2021;7(1):dvab013.
50. Howe CG, Cox B, Fore R, Jungius J, Kvist T, Lent S, et al. Maternal Gestational Diabetes Mellitus and Newborn DNA Methylation: Findings From the Pregnancy and Childhood Epigenetics Consortium. Diabetes Care. 2019;43(1):98-105.
51. Finer S, Mathews C, Lowe R, Smart M, Hillman S, Foo L, et al. Maternal gestational diabetes is associated with genome-wide DNA methylation variation in placenta and cord blood of exposed offspring. Human Molecular Genetics. 2015;24(11):3021-9.
52. Haertle L, El Hajj N, Dittrich M, Müller T, Nanda I, Lehnen H, et al. Epigenetic signatures of gestational diabetes mellitus on cord blood methylation. Clinical Epigenetics. 2017;9(1):28.
53. Awamleh Z, Butcher DT, Hanley A, Retnakaran R, Haertle L, Haaf T, et al. Exposure to Gestational Diabetes Mellitus (GDM) alters DNA methylation in placenta and fetal cord blood. Diabetes Research and Clinical Practice. 2021;174:108690.
54. Słupecka-Ziemilska M, Wychowański P, Puzianowska-Kuznicka M. Gestational Diabetes Mellitus Affects Offspring's Epigenome. Is There a Way to Reduce the Negative Consequences? Nutrients. 2020;12(9):2792.
55. Ben-Shlomo Y, Mishra GD, Kuh D. Life course epidemiology. Handbook of epidemiology: Springer; 2023. p. 1-31.
56. Ben-Shlomo Y, Cooper R, Kuh D. The last two decades of life course epidemiology, and its relevance for research on ageing. International journal of epidemiology. 2016;45(4):973-88.

57. Song P, Zhang Y, Yu J, Zha M, Zhu Y, Rahimi K, et al. Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. JAMA Pediatrics. 2019;173(12):1154-63.
58. Sharma AK, Metzger DL, Rodd CJ. Prevalence and Severity of High Blood Pressure Among Children Based on the 2017 American Academy of Pediatrics Guidelines. JAMA Pediatr. 2018;172(6):557-65.
59. Dart A, Singer A, Chanchlani R, Ferguson T, Tangri N, Zappitelli M. Evaluation of administrative case definitions for hypertension in Canadian children. Sci Rep. 2023;13(1):7654.
60. Bell CS, Samuel JP, Samuels JA. Prevalence of Hypertension in Children. Hypertension (Dallas, Tex : 1979). 2019;73(1):148-52.
61. Robinson SK, Rodd CJ, Metzger DL, Sharma AK. Prevalence of high blood pressure among Canadian Children: 2017 American Academy of Pediatrics guidelines with the Canadian Health Measures Survey. Paediatrics & Child Health. 2020;26(3):e158-e65.
62. Lande MB, Carson NL, Roy J, Meagher CC. Effects of childhood primary hypertension on carotid intima media thickness: a matched controlled study. Hypertension (Dallas, Tex : 1979). 2006;48(1):40-4.
63. Robinson CH, Hussain J, Jeyakumar N, Smith G, Birken CS, Dart A, et al. Long-Term Cardiovascular Outcomes in Children and Adolescents With Hypertension. JAMA Pediatr. 2024;178(7):688-98.
64. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics. 2017;140(3).
65. Ding L, Singer A, Kosowan L, Dart A. Pediatric hypertension screening and recognition in primary care clinics in Canada. Paediatrics & Child Health. 2022;27(2):118-26.
66. Yang L, Kelishadi R, Hong YM, Khadilkar A, Nawarycz T, Krzywińska-Wiewiorowska M, et al. Impact of the 2017 American Academy of Pediatrics Guideline on Hypertension Prevalence Compared With the Fourth Report in an International Cohort. Hypertension (Dallas, Tex : 1979). 2019;74(6):1343-8.
67. Naidoo S, Kagura J, Fabian J, Norris SA. Early Life Factors and Longitudinal Blood Pressure Trajectories Are Associated With Elevated Blood Pressure in Early Adulthood. Hypertension (Dallas, Tex : 1979). 2019;73(2):301-9.
68. Guzman-Limon M, Samuels J. Pediatric hypertension: diagnosis, evaluation, and treatment. Pediatric Clinics. 2019;66(1):45-57.
69. Bucher BS, Ferrarini A, Weber N, Bullo M, Bianchetti MG, Simonetti GD. Primary Hypertension in Childhood. Current Hypertension Reports. 2013;15(5):444-52.
70. Raina R, Mahajan Z, Sharma A, Chakraborty R, Mahajan S, Sethi SK, et al. Hypertensive Crisis in Pediatric Patients: An Overview. Frontiers in Pediatrics. 2020;8.
71. Feig DS, Artani A, Asaf A, Li P, Booth GL, Shah BR. Long-term Neurobehavioral and Metabolic Outcomes in Offspring of Mothers With Diabetes During Pregnancy: A Large, Population-Based Cohort Study in Ontario, Canada. Diabetes Care. 2024.
72. Pathirana MM, Lassi ZS, Roberts CT, Andraweera PH. Cardiovascular risk factors in offspring exposed to gestational diabetes mellitus in utero: systematic review and meta-analysis. Journal of Developmental Origins of Health and Disease. 2020;11(6):599-616.
73. Ravelli G-P, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. New England Journal of Medicine. 1976;295(7):349-53.

74. Tam WH, Ma RC, Yip GW, Yang X, Li AM, Ko GT, et al. The association between in utero hyperinsulinemia and adolescent arterial stiffness. Diabetes Res Clin Pract. 2012;95(1):169-75.
75. Aris IM, Rifas-Shiman SL, Li LJ, Belfort MB, Hivert MF, Oken E. Early-Life Predictors of Systolic Blood Pressure Trajectories From Infancy to Adolescence: Findings From Project Viva. Am J Epidemiol. 2019;188(11):1913-22.
76. Bianco ME, Josefson JL. Hyperglycemia During Pregnancy and Long-Term Offspring Outcomes. Curr Diab Rep. 2019;19(12):143.
77. Gillman MW. Developmental origins of health and disease. N Engl J Med. 2005;353(17):1848-50.
78. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia. 1993;36(1):62-7.
79. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet (London, England). 1993;341(8850):938-41.
80. Habib S, Gattineni J, Twombley K, Baum M. Evidence that prenatal programming of hypertension by dietary protein deprivation is mediated by fetal glucocorticoid exposure. American journal of hypertension. 2011;24(1):96-101.
81. Burrage D, Green L, Moss T, Sloboda DM, Nitsos I, Newnham J, et al. The carotid bodies influence growth responses to moderate maternal undernutrition in late-gestation fetal sheep. BJOG: An International Journal of Obstetrics & Gynaecology. 2008;115(2):261-8.
82. Gademan MG, van Eijsden M, Roseboom TJ, van der Post JA, Stronks K, Vrijkotte TG. Maternal prepregnancy body mass index and their children's blood pressure and resting cardiac autonomic balance at age 5 to 6 years. Hypertension (Dallas, Tex : 1979). 2013;62(3):641-7.
83. Ludwig-Walz H, Schmidt M, Gunther ALB, Kroke A. Maternal prepregnancy BMI or weight and offspring's blood pressure: Systematic review. Matern Child Nutr. 2018;14(2):e12561.
84. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med. 2017;377(1):13-27.
85. Basu M, Garg V. Maternal hyperglycemia and fetal cardiac development: Clinical impact and underlying mechanisms. Birth Defects Res. 2018;110(20):1504-16.
86. Morton JS, Cooke CL, Davidge ST. In Utero Origins of Hypertension: Mechanisms and Targets for Therapy. Physiological reviews. 2016;96(2):549-603.
87. Dorey ES, Pantaleon M, Weir KA, Moritz KM. Adverse prenatal environment and kidney development: implications for programing of adult disease. Reproduction. 2014;147(6):R189-98.
88. Turdi S, Ge W, Hu N, Bradley KM, Wang X, Ren J. Interaction between maternal and postnatal high fat diet leads to a greater risk of myocardial dysfunction in offspring via enhanced lipotoxicity, IRS-1 serine phosphorylation and mitochondrial defects. J Mol Cell Cardiol. 2013;55:117-29.
89. Mdaki KS, Larsen TD, Wachal AL, Schimelpfenig MD, Weaver LJ, Dooyema SD, et al. Maternal high-fat diet impairs cardiac function in offspring of diabetic pregnancy through metabolic stress and mitochondrial dysfunction. Am J Physiol Heart Circ Physiol. 2016;310(6):H681-92.

90. Armitage JA, Taylor PD, Poston L. Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. J Physiol. 2005;565(Pt 1):3-8.
91. Hoodbhoy Z, Mohammed N, Aslam N, Fatima U, Ashiqali S, Rizvi A, et al. Is the child at risk? Cardiovascular remodelling in children born to diabetic mothers. Cardiol Young. 2019;29(4):467-74.
92. Rijpert M, Breur JM, Evers IM, de Valk HW, Heijnen CJ, Meijboom FJ, et al. Cardiac function in 7-8-year-old offspring of women with type 1 diabetes. Exp Diabetes Res. 2011;2011:564316.
93. Guillemette L, Dart A, Wicklow B, Dolinsky VW, Cheung D, Jassal DS, et al. Cardiac structure and function in youth with type 2 diabetes in the iCARE cohort study: Cross-sectional associations with prenatal exposure to diabetes and metabolomic profiles. Pediatr Diabetes. 2020;21(2):233-42.
94. Chehade H, Simeoni U, Guignard JP, Boubred F. Preterm Birth: Long Term Cardiovascular and Renal Consequences. Curr Pediatr Rev. 2018;14(4):219-26.
95. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis--2012 Curt Richter Award Winner. Psychoneuroendocrinology. 2013;38(1):1-11.
96. Brion M-JA, Leary SD, Lawlor DA, Smith GD, Ness AR. Modifiable maternal exposures and offspring blood pressure: a review of epidemiological studies of maternal age, diet, and smoking. Pediatric research. 2008;63(6):593-8.
97. Schütte T, Kedziora SM, Haase N, Herse F, Busjahn A, Birukov A, et al. Intrauterine Exposure to Diabetic Milieu Does Not Induce Diabetes and Obesity in Male Adulthood in a Novel Rat Model. Hypertension (Dallas, Tex : 1979). 2020:Hypertensionaha12016360.
98. Litwin L, Sundholm JK, Rönö K, Koivusalo SB, Eriksson JG, Sarkola T. No effect of gestational diabetes or pre-gestational obesity on 6-year offspring left ventricular function—RADIEL study follow-up. Acta diabetologica. 2020;57(12):1463-72.
99. Leon DA. Twins and fetal programming of blood pressure: questioning the role of genes and maternal nutrition. British Medical Journal Publishing Group; 1999.
100. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? The Lancet. 2002;360(9334):659-65.
101. Tu Y-K, West R, Ellison GT, Gilthorpe MS. Why evidence for the fetal origins of adult disease might be a statistical artifact: the “reversal paradox” for the relation between birth weight and blood pressure in later life. American journal of epidemiology. 2005;161(1):27-32.
102. Brunton NM, Dufault B, Dart A, Azad MB, McGavock JM. Maternal body mass index, offspring body mass index, and blood pressure at 18 years: a causal mediation analysis. Int J Obes (Lond). 2021.
103. Cole SR, Hernán MA. Fallibility in estimating direct effects. International journal of epidemiology. 2002;31(1):163-5.
104. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. 1999;10(1):37-48.
105. Greenland S, Pearl J, Robins JM. Confounding and collapsibility in causal inference. Statistical science. 1999;14(1):29-46.
106. Hernán MA, Robins JM. Estimating causal effects from epidemiological data. Journal of Epidemiology & Community Health. 2006;60(7):578-86.

107. Rubin DB. Causal Inference Using Potential Outcomes. Journal of the American Statistical Association. 2005;100(469):322-31.
108. Rubin DB. Comment: Which ifs have causal answers. Journal of the American statistical association. 1986;81(396):961-2.
109. Pearl J. Causality: Cambridge university press; 2009.
110. Pearl J. Graphs, causality, and structural equation models. Sociological Methods & Research. 1998;27(2):226-84.
111. Pearl J. An introduction to causal inference. The international journal of biostatistics. 2010;6(2).
112. Rohrer JM. Thinking clearly about correlations and causation: Graphical causal models for observational data. Advances in methods and practices in psychological science. 2018;1(1):27-42.
113. Holland PW, Glymour C, Granger C. STATISTICS AND CAUSAL INFERENCE. ETS research report series. 1985;1985(2):i-72.
114. Rubin DB. Randomization Analysis of Experimental Data: The Fisher Randomization Test Comment. Journal of the American Statistical Association. 1980;75(371):591-3.
115. Morgan SL, Winship C. Counterfactuals and causal inference: Cambridge University Press; 2015.
116. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health. 2005;95 Suppl 1:S144-50.
117. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. International Journal of Epidemiology. 2017;45(6):1866-86.
118. Caramaschi D, Taylor AE, Richmond RC, Havdahl KA, Golding J, Relton CL, et al. Maternal smoking during pregnancy and autism: using causal inference methods in a birth cohort study. Translational Psychiatry. 2018;8(1):262.
119. Lund IO, Hannigan LJ, Ask H, Askelund A, Hegemann L, Corfield EC, et al. Prenatal maternal stress: triangulating evidence for intrauterine exposure effects on birth and early childhood outcomes across multiple approaches. 2024.
120. Borges MC, Clayton GL, Freathy RM, Felix JF, Fernández-Sanlés A, Soares AG, et al. Integrating multiple lines of evidence to assess the effects of maternal BMI on pregnancy and perinatal outcomes. BMC medicine. 2024;22(1):32.
121. Gyasi-Antwi P, Walker L, Moody C, Okyere S, Salt K, Anang L, et al. Global prevalence of gestational diabetes mellitus: a systematic review and meta-analysis. New American Journal of Medicine. 2020;1(3).
122. Canada PHAo. Maternal Diabetes in Canada. 2018.
123. Lipscombe LL, Hux JE. Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995–2005: a population-based study. The Lancet. 2007;369(9563):750-6.
124. Ngwezi DP, Savu A, Yeung RO, Butalia S, Kaul P. Temporal Trends in Type 1, Type 2, and Gestational Diabetes in Pregnancy: Impact of Rural Residence, Ethnicity, and Material Deprivation. Canadian Journal of Diabetes. 2023.
125. Nethery E, Law MR, Kotaska A, Janssen PA, Hutcheon JA. The effect of changing screening practices and demographics on the incidence of gestational diabetes in British Columbia, 2005–2019. CMAJ. 2023;195(11):E396-E403.
126. Aljohani N, Rempel BM, Ludwig S, Morris M, McQuillen K, Cheang M, et al. Gestational diabetes in Manitoba during a twenty-year period. Clinical and Investigative Medicine. 2008:E131-E7.

127. Lawrence JM, Contreras R, Chen W, Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999-2005. Diabetes Care. 2008;31(5):899-904.
128. Association AD. Gestational diabetes mellitus. Diabetes care. 2004;27:S88.
129. Behboudi-Gandevani S, Amiri M, Bidhendi Yarandi R, Ramezani Tehrani F. The impact of diagnostic criteria for gestational diabetes on its prevalence: a systematic review and meta-analysis. Diabetology & metabolic syndrome. 2019;11(1):1-18.
130. Peticca P, Keely EJ, Walker MC, Yang Q, Bottomley J. Pregnancy outcomes in diabetes subtypes: how do they compare? A province-based study of Ontario, 2005–2006. Journal of Obstetrics and Gynaecology Canada. 2009;31(6):487-96.
131. Metcalfe A, Sabr Y, Hutcheon JA, Donovan L, Lyons J, Burrows J, et al. Trends in Obstetric Intervention and Pregnancy Outcomes of Canadian Women With Diabetes in Pregnancy From 2004 to 2015. Journal of the Endocrine Society. 2017;1(12):1540-9.
132. Sweeting A, Wong J, Murphy HR, Ross GP. A Clinical Update on Gestational Diabetes Mellitus. Endocrine Reviews. 2022;43(5):763-93.
133. Bellamy L, Casas J-P, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. The Lancet. 2009;373(9677):1773-9.
134. Song C, Lyu Y, Li C, Liu P, Li J, Ma R, et al. Long-term risk of diabetes in women at varying durations after gestational diabetes: a systematic review and meta-analysis with more than 2 million women. Obesity reviews. 2018;19(3):421-9.
135. Kramer CK, Campbell S, Retnakaran R. Gestational diabetes and the risk of cardiovascular disease in women: a systematic review and meta-analysis. Diabetologia. 2019;62:905-14.
136. Chu AH, Godfrey KM. Gestational diabetes mellitus and developmental programming. Annals of Nutrition and Metabolism. 2021;76(Suppl. 3):4-15.
137. Fraser A, Lawlor DA. Long-term health outcomes in offspring born to women with diabetes in pregnancy. Current diabetes reports. 2014;14:1-8.
138. Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, et al. Screening for Gestational Diabetes: US Preventive Services Task Force Recommendation Statement. Jama. 2021;326(6):531-8.
139. Katz A, Enns J, Smith M, Burchill C, Turner K, Towns D. Population Data Centre Profile: The Manitoba Centre for Health Policy. Int J Popul Data Sci. 2020;4(2):1131.
140. Heaman M, Kingston D, Helewa ME, Brownell M, Derksen S, Bogdanovic B, et al. Perinatal services and outcomes in Manitoba. Winnipeg, MB: Manitoba Centre for Health Policy. 2012;20.
141. Jutte DP, Roos LL, Brownell MD. Administrative record linkage as a tool for public health research. Annual review of public health. 2011;32:91-108.
142. Roos LL, Nicol JP. A research registry: uses, development, and accuracy. Journal of clinical epidemiology. 1999;52(1):39-47.
143. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet (London, England). 2007;370(9596):1453-7.
144. Lesko CR, Fox MP, Edwards JK. A Framework for Descriptive Epidemiology. Am J Epidemiol. 2022;191(12):2063-70.

145. Dart AB, Ruth CA, Sellers EA, Au W, Dean HJ. Maternal diabetes mellitus and congenital anomalies of the kidney and urinary tract (CAKUT) in the child. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2015;65(5):684-91.
146. Shen GX, Shafer LA, Martens PJ, Sellers E, Torshizi AA, Ludwig S, et al. Does First Nations ancestry modify the association between gestational diabetes and subsequent diabetes: a historical prospective cohort study among women in Manitoba, Canada. Diabetic medicine : a journal of the British Diabetic Association. 2016;33(9):1245-52.
147. Shah BR, Booth GL, Feig DS, Lipscombe LL. Validation of algorithms to identify gestational diabetes from population-level health-care administrative data. Canadian Journal of Diabetes. 2023;47(1):25-30.
148. Ngwezi DP, Savu A, Yeung RO, Butalia S, Kaul P. Validity of alternative claims-based algorithms for type 1, type 2, and gestational diabetes in pregnancy. Canadian Journal of Diabetes. 2023;47(8):643-8. e1.
149. Bowker S, Savu A, Donovan L, Johnson J, Kaul P. Validation of administrative and clinical case definitions for gestational diabetes mellitus against laboratory results. Diabetic Medicine. 2017;34(6):781-5.
150. Wicklow BA, Sellers EAC, Sharma AK, Kroeker K, Nickel NC, Philips-Beck W, et al. Association of Gestational Diabetes and Type 2 Diabetes Exposure In Utero With the Development of Type 2 Diabetes in First Nations and Non-First Nations Offspring. JAMA Pediatr. 2018;172(8):724-31.
151. Kim SY, Sharma AJ, Sappenfield W, Wilson HG, Salihu HM. Association of maternal body mass index, excessive weight gain, and gestational diabetes mellitus with large-for-gestational-age births. Obstet Gynecol. 2014;123(4):737-44.
152. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991-2002.
153. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med. 2009;361(14):1339-48.
154. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005;352(24):2477-86.
155. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr. 2013;13:59.
156. Kramer MS, Platt RW, Wen SW, Joseph KS, Allen A, Abrahamowicz M, et al. A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics. 2001;108(2):E35.
157. Chateau D, Metge C, Prior H, Soodeen R-A. Learning from the census: the Socio-economic Factor Index (SEFI) and health outcomes in Manitoba. Canadian Journal of Public Health/Revue Canadienne De Sante'E Publique. 2012:S23-S7.
158. Fransoo R, Prior H, Allegro E, Burchill CA, Bailly A, Koseva I, et al. The 2013 RHA indicators atlas: desLibris; 2013.
159. Getahun D, Fassett MJ, Jacobsen SJ. Gestational diabetes: risk of recurrence in subsequent pregnancies. American journal of obstetrics and gynecology. 2010;203(5):467. e1-. e6.

160. Feig DS, Hwee J, Shah BR, Booth GL, Bierman AS, Lipscombe LL. Trends in Incidence of Diabetes in Pregnancy and Serious Perinatal Outcomes: A Large, Population-Based Study in Ontario, Canada, 1996–2010. Diabetes Care. 2014;37(6):1590-6.
161. Roustaei Z, Anttonen S, Räisänen S, Gissler M, Heinonen S. Socioeconomic status, maternal risk factors, and gestational diabetes mellitus across reproductive years: a Finnish register-based study. BMJ Open Diabetes Research & Care. 2023;11(4):e003278.
162. Li Y, Ren X, He L, Li J, Zhang S, Chen W. Maternal age and the risk of gestational diabetes mellitus: A systematic review and meta-analysis of over 120 million participants. Diabetes Res Clin Pract. 2020;162:108044.
163. Marmot M. Health in an unequal world. The Lancet. 2006;368(9552):2081-94.
164. Lemstra M, Neudorf C, Opondo J. Health disparity by neighbourhood income. Canadian Journal of Public Health. 2006;97:435-9.
165. Kivimäki M, Batty GD, Pentti J, Shipley MJ, Sipilä PN, Nyberg ST, et al. Association between socioeconomic status and the development of mental and physical health conditions in adulthood: a multi-cohort study. The Lancet Public Health. 2020;5(3):e140-e9.
166. Martens PJ, Fransoo R, Burchill C, Burland E. Health status and healthcare use patterns of rural, northern and urban Manitobans: is Romanow right? Healthcare Policy. 2006;2(1):108.
167. Lavoie JG, Philips-Beck W, Kinew KA, Kyoon-Achan G, Sinclair S, Katz A. The relationship between rates of hospitalization for ambulatory care sensitive conditions and local access to primary healthcare in Manitoba First Nations communities. Canadian Journal of Public Health. 2021;112(2):219-30.
168. Urquia M, Prior H, Detillieux G, Eze ND, Koseva LI. The diversity of Immigrants to Manitoba: Migration dynamics and basic healthcare service use. Manitoba Centre for Health Policy, Winnipeg. http://mchp-appserv. cpe …; 2020.
169. Shah NS, Wang MC, Freaney PM, Perak AM, Carnethon MR, Kandula NR, et al. Trends in Gestational Diabetes at First Live Birth by Race and Ethnicity in the US, 2011-2019. JAMA. 2021;326(7):660-9.
170. Ramezani Tehrani F, Naz MSG, Yarandi RB, Behboudi-Gandevani S. The impact of diagnostic criteria for gestational diabetes mellitus on adverse maternal outcomes: a systematic review and meta-analysis. Journal of clinical medicine. 2021;10(4):666.
171. Farrar D, Fairley L, Wright J, Tuffnell D, Whitelaw D, Lawlor DA. Evaluation of the impact of universal testing for gestational diabetes mellitus on maternal and neonatal health outcomes: a retrospective analysis. BMC Pregnancy and Childbirth. 2014;14(1):317.
172. Pouliot A, Elmahboubi R, Adam C. Incidence and outcomes of gestational diabetes mellitus using the new International Association of Diabetes in Pregnancy Study Group Criteria in Hôpital Maisonneuve-Rosemont. Canadian Journal of Diabetes. 2019;43(8):594-9.
173. Yu Y, Arah OA, Liew Z, Cnattingius S, Olsen J, Sørensen HT, et al. Maternal diabetes during pregnancy and early onset of cardiovascular disease in offspring: population based cohort study with 40 years of follow-up. BMJ. 2019;367:l6398.
174. Beyerlein A, Nehring I, Rosario AS, von Kries R. Gestational diabetes and cardiovascular risk factors in the offspring: Results from a cross-sectional study. Diabetic medicine. 2012;29(3):378-84.
175. Tam WH, Ma RCW, Yang X, Li AM, Ko GTC, Kong APS, et al. Glucose intolerance and cardiometabolic risk in adolescents exposed to maternal gestational diabetes: a 15-year follow-up study. Diabetes care. 2010;33(6):1382-4.

176. Frach L, Jami ES, McAdams TA, Dudbridge F, Pingault J-B. Causal inference methods for intergenerational research using observational data. Psychological Review. 2023;130(6):1688-703.
177. Brion MJ, Lawlor DA, Matijasevich A, Horta B, Anselmi L, Araújo CL, et al. What are the causal effects of breastfeeding on IQ, obesity and blood pressure? Evidence from comparing high-income with middle-income cohorts. Int J Epidemiol. 2011;40(3):670-80.
178. Guillemette L, Wicklow B, Sellers EA, Dart A, Shen GX, Dolinsky VW, et al. Intrauterine exposure to diabetes and risk of cardiovascular disease in adolescence and early adulthood: a population-based birth cohort study. CMAJ. 2020;192(39):E1104-E13.
179. Haseler E, Sinha MD. Hypertension in children and young adults. Pediatric Clinics. 2022;69(6):1165-80.
180. Ruth C. Type 2 diabetes in Manitoba: Manitoba Centre for Health Policy; 2020.
181. Iacus SM, King G, Porro G. A Theory of Statistical Inference for Matching Methods in Causal Research. Political analysis. 2019;27(1):46-68.
182. Stuart EA. Matching methods for causal inference: A review and a look forward. Stat Sci. 2010;25(1):1-21.
183. Godfrey KE. Creating Matched Samples Using Exact Matching. Statistical Report 2016-3. College Board. 2016.
184. Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. Epidemiology. 2010;21(3):383-8.
185. Chen Q, Sjölander A, Långström N, Rodriguez A, Serlachius E, D'Onofrio BM, et al. Maternal pre-pregnancy body mass index and offspring attention deficit hyperactivity disorder: a population-based cohort study using a sibling-comparison design. International journal of epidemiology. 2014;43(1):83-90.
186. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. Diabetes. 2000;49(12):2208-11.
187. Lawlor DA, Lichtenstein P, Langstrom N. Association of maternal diabetes mellitus in pregnancy with offspring adiposity into early adulthood: sibling study in a prospective cohort of 280,866 men from 248,293 families. Circulation. 2011;123(3):258-65.
188. Razaz N, Villamor E, Muraca GM, Bonamy A-KE, Cnattingius S. Maternal obesity and risk of cardiovascular diseases in offspring: a population-based cohort and sibling-controlled study. The lancet Diabetes & endocrinology. 2020;8(7):572-81.
189. Sellers EA, Dean HJ, Shafer LA, Martens PJ, Phillips-Beck W, Heaman M, et al. Exposure to Gestational Diabetes Mellitus: Impact on the Development of Early-Onset Type 2 Diabetes in Canadian First Nations and Non-First Nations Offspring. Diabetes Care. 2016;39(12):2240-6.
190. Fraser A, Almqvist C, Larsson H, Långström N, Lawlor DA. Maternal diabetes in pregnancy and offspring cognitive ability: sibling study with 723,775 men from 579,857 families. Diabetologia. 2014;57(1):102-9.
191. Frisell T, Öberg S, Kuja-Halkola R, Sjölander A. Sibling comparison designs: Bios from non-shored confounders and measurement error. Epidemiology. 2012:713-20.
192. Lu J, Zhang S, Li W, Leng J, Wang L, Liu H, et al. Maternal Gestational Diabetes Is Associated With Offspring's Hypertension. American Journal of Hypertension. 2019;32(4):335-42.

193. Eletri L, Mitanchez D. How do the different types of maternal diabetes during pregnancy influence offspring outcomes? Nutrients. 2022;14(18):3870.
194. Aceti A, Santhakumaran S, Logan KM, Philipps LH, Prior E, Gale C, et al. The diabetic pregnancy and offspring blood pressure in childhood: a systematic review and meta-analysis. Diabetologia. 2012;55(11):3114-27.
195. Tocantins C, Diniz MS, Grilo LF, Pereira SP. The birth of cardiac disease: Mechanisms linking gestational diabetes mellitus and early onset of cardiovascular disease in offspring. WIREs mechanisms of disease. 2022;14(4):e1555.
196. Bianco ME, Josefson JL. Hyperglycemia during pregnancy and long-term offspring outcomes. Current diabetes reports. 2019;19:1-8.
197. Battin MR, Obolonkin V, Rush E, Hague W, Coat S, Rowan J. Blood pressure measurement at two years in offspring of women randomized to a trial of metformin for GDM: follow up data from the MiG trial. BMC Pediatr. 2015;15:54.
198. Landon MB, Mele L, Varner MW, Casey BM, Reddy UM, Wapner RJ, et al. The relationship of maternal glycemia to childhood obesity and metabolic dysfunction. The Journal of Maternal-Fetal & Neonatal Medicine. 2020;33(1):33-41.
199. Kyriacou DN, Lewis RJ. Confounding by Indication in Clinical Research. JAMA. 2016;316(17):1818-9.
200. Dabelea D. The predisposition to obesity and diabetes in offspring of diabetic mothers. Diabetes care. 2007;30(Supplement_2):S169-S74.
201. Barker DJ, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. J Epidemiol Community Health. 1989;43(3):237-40.
202. Moen G-H, Brumpton B, Willer C, Åsvold BO, Birkeland KI, Wang G, et al. Mendelian randomization study of maternal influences on birthweight and future cardiometabolic risk in the HUNT cohort. Nature Communications. 2020;11(1):5404.
203. Hwang L-D, Lawlor DA, Freathy RM, Evans DM, Warrington NM. Using a two-sample Mendelian randomization design to investigate a possible causal effect of maternal lipid concentrations on offspring birth weight. International journal of epidemiology. 2019;48(5):1457-67.
204. Weinert LS. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy: comment to the International Association of Diabetes and Pregnancy Study Groups Consensus Panel. Diabetes care. 2010;33(7):e97-e.
205. Cole SR, Frangakis CE. The Consistency Statement in Causal Inference: A Definition or an Assumption? Epidemiology. 2009;20(1):3-5.
206. Rehkopf DH, Glymour MM, Osypuk TL. The Consistency Assumption for Causal Inference in Social Epidemiology: When a Rose is Not a Rose. Curr Epidemiol Rep. 2016;3(1):63-71.
207. Agarwal M, Dhatt G, Othman Y. Gestational diabetes mellitus prevalence: Effect of the laboratory analytical variation. Diabetes Research and Clinical Practice. 2015;109(3):493-9.
208. Sharp GC, Schellhas L, Richardson SS, Lawlor DA. Time to cut the cord: recognizing and addressing the imbalance of DOHaD research towards the study of maternal pregnancy exposures. Journal of Developmental Origins of Health and Disease. 2019;10(5):509-12.

209. Field C, Grobman WA, Yee LM, Johnson J, Wu J, McNeil B, et al. Community-level social determinants of health and pregestational and gestational diabetes. American Journal of Obstetrics & Gynecology MFM. 2024;6(2):101249.
210. Zaidi AH, Brewer B, Zadokar V, Lopes J, Witherell C, Miller J, et al. High Prevalence of Pediatric Hypertension is Associated With Social Determinants of Health Including Childhood Opportunity Index. Circulation. 2023;148(Suppl_1):A18937-A.
211. Richardson SS, Daniels CR, Gillman MW, Golden J, Kukla R, Kuzawa C, et al. Society: Don't blame the mothers. Nature. 2014;512(7513):131-2.

Chapter 9: Supplementary Material

9.1 Chapter 4 Supplements

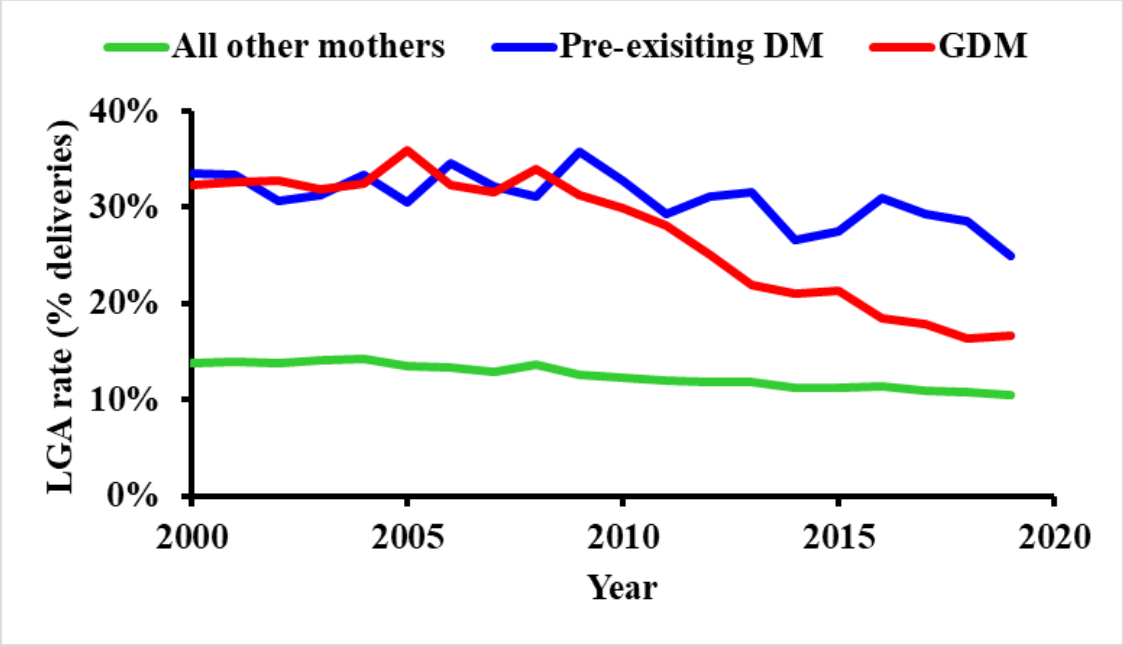

Figure 9.1 Incidence of large for gestational age infants born in Manitoba from 2000 – 2019. GDM, gestational diabetes mellitus; DM, diabetes mellitus, LGA, large for gestational age.

Table 9.1 Descending gestational diabetes incidence and neighbourhood area characteristics based on data from 2016 Canadian Census.

Neighbourhood Area	Gestational diabetes Incidence	Unemployment rate for labour force 15+ years	% Visible Minorities	% immigrated last 10 years	% 15+ years without High School graduation
08A	7.3-8.8	6.22 ± 3.57	0.58 ± 0.16	0.26 ± 0.13	0.19 ± 0.06
08B	7.3-8.8	5.73 ± 3.22	0.31 ± 0.13	0.15 ± 0.08	0.18 ± 0.06
09A	7.3-8.8	6.41 ± 3.94	0.63 ± 0.11	0.23 ± 0.1	0.19 ± 0.06
09B	7.3-8.8	7.2 ± 4.53	0.47 ± 0.16	0.26 ± 0.12	0.27 ± 0.07
03B	6.4-7.3	6.64 ± 4.00	0.41 ± 0.20	0.16 ± 0.1	0.11 ± 0.04
07C	6.4-7.3	7.49 ± 3.28	0.26 ± 0.12	0.14 ± 0.09	0.18 ± 0.07
10A	6.4-7.3	8.93 ± 4.49	0.37 ± 0.17	0.2 ± 0.12	0.27 ± 0.08
11B	6.4-7.3	10.24 ± 4.99	0.44 ± 0.17	0.25 ± 0.12	0.22 ± 0.13
01B	5.3-6.4	4.88 ± 3.45	0.17 ± 0.09	0.06 ± 0.06	0.13 ± 0.08
04A	5.3-6.4	6.62 ± 3.97	0.17 ± 0.13	0.09 ± 0.1	0.15 ± 0.06
04B	5.3-6.4	5.3 ± 3.39	0.2 ± 0.09	0.08 ± 0.05	0.13 ± 0.05
05A	5.3-6.4	6.43 ± 2.98	0.13 ± 0.07	0.06 ± 0.05	0.15 ± 0.07
006	5.3-6.4	6.74 ± 2.86	0.16 ± 0.12	0.08 ± 0.07	0.19 ± 0.06
07A	5.3-6.4	8.56 ± 4.21	0.22 ± 0.1	0.1 ± 0.06	0.24 ± 0.07
10B	5.3-6.4	12.89 ± 6.88	0.24 ± 0.14	0.12 ± 0.09	0.37 ± 0.13
11A	5.3-6.4	6.74 ± 3.08	0.27 ± 0.19	0.11 ± 0.09	0.17 ± 0.08
01A	4.4-5.3	5.4 ± 2.77	0.09 ± 0.05	0.04 ± 0.03	0.15 ± 0.06
002	4.4-5.3	4.93 ± 3.89	0.1 ± 0.06	0.04 ± 0.05	0.11 ± 0.04
03A	4.4-5.3	6.29 ± 3.64	0.23 ± 0.13	0.09 ± 0.07	0.12 ± 0.05
05B	4.4-5.3	5.12 ± 2.59	0.19 ± 0.09	0.07 ± 0.05	0.15 ± 0.05
07B	4.4-5.3	5.25 ± 2.41	0.11 ± 0.08	0.05 ± 0.05	0.18 ± 0.06
08C	4.4-5.3	3.31 ± 2.37	0.09 ± 0.07	0.03 ± 0.03	0.17 ± 0.02
12B	4.4-5.3	6.75 ± 3.39	0.15 ± 0.1	0.06 ± 0.06	0.1 ± 0.06
07D	3.8-4.4	4.55 ± 1.72	0.05 ± 0.06	0.01 ± 0.03	0.13 ± 0.04
12A	3.8-4.4	5.14 ± 3.04	0.15 ± 0.12	0.08 ± 0.08	0.11 ± 0.04

9.2 Chapter 5 Supplements

Table 9.2 Description of databases used to create the administrative birth cohorts

Data Source	Description
Manitoba Health Insurance Registry	Contains longitudinal data on all Manitobans who have been registered with Manitoba Health at some point since 1970. The registry includes individual-level demographics, family composition information, residential postal codes, and data fields for registration, birth, entry into province, and migration in/out of province.
Hospital Abstracts	Summaries of demographic and clinical information (e.g., gender, diagnoses, and procedure codes) completed upon discharge from the hospital.
Medical Claims	Health data consisting of claims for physician visits in offices, hospitals, and outpatient departments. In Manitoba, fee-for-service providers must submit claims to Manitoba Health for reimbursement.
Drug Program Information Network	An electronic, on-line, point-of-sale prescription drug database that connects Manitoba Health and all pharmacies in Manitoba. This system generates complete drug profiles for each client including all transactions at the point of distribution.
Diabetes Education Resource for Children and Adolescents	Contains health information on children and youth under the age of 18 who are diagnosed with youth onset Type 1 or Type 2 Diabetes in Manitoba.
Diagnostic Services Manitoba	Includes records of Manitoba's Hospital laboratory services from 2006 – 2016.
Census	The Canadian census contains aggregate demographic information (e.g., age, sex, marital status, employment, and income) for all persons via residential six-digit postal codes.

Table 9.3 Disease classification for primary exposure and outcome with corresponding International Classification of Disease (ICD) codes.

Indication	ICD Title	ICD 10 Code	ICD 9 Code
Diagnoses used in defining maternal diabetes (Primary Exposure)			
Gestational Diabetes	Diabetes mellitus in pregnancy, childbirth, and the puerperium	O24, P70.0	648.8, 648.0
	Malnutrition – related diabetes	E12	250
Other Diabetes	Other specified diabetes mellitus	E13, R73.0	NA
	Unspecified diabetes mellitus	E14	NA
Type 2 Diabetes	Type 2 diabetes mellitus	E11, O24.1, O24.6	250.x0, 250.x2
Type 1 Diabetes	Type 1 diabetes mellitus	E10, O24.5	250.x1, 250.x3
Diagnoses used in defining offspring hypertension (Primary Outcome)			
Primary hypertension	Essential (primary) hypertension	I10	401
	Hypertensive heart disease	I11	402
	Hypertensive renal disease	I12	403
	Hypertensive heart and renal disease	I13	404
Secondary hypertension	Renovascular hypertension	I15.0	405.1
	Hypertension secondary to other renal disorders	I15.1	405.91
	Hypertension secondary to endocrine disorders	I15.2	405.19
	Other secondary hypertension	I15.8	405.19
	Secondary hypertension, unspecified	I15.9	405.99
Diagnoses leading to exclusion from the cohort			
Cardiomyopathy	Endocardial fibroelastosis	I42.4	425.3
	Endomyocardial disease	I42.3	425.0
	Congenital cardiomyopathy	I42.8	425.9
	Familial cardiomyopathy	I42.9	425.9
Congenital malformation of circulatory system	Congenital malformations of the circulatory system	Q20 – Q 28	745-747, 759.9
Cystic fibrosis	Cystic fibrosis	E84	277.0

Table 9.4 Definitions for Disease Diagnosis

Disease	Definition
Gestational Diabetes	1+ hospitalizations with diagnosis code for incident diabetes at 21+ weeks gestation (Table S2) • Incident diabetes diagnosis at 21+ weeks gestation, as this is likely miss-coded gestational diabetes, provided that there are no "regular" diabetes diagnoses in the 6 weeks after birth. Notes: • If the gestation period is missing, too high, or too low, a 9-month gestation period is assumed. • Additional (non-incident) diabetes coded in the 6-week period after birth is an indication of pre-existing diabetes. • Women with pre-existing diabetes prior to 21 weeks gestation were considered to not have gestational diabetes.
Type 1 Diabetes	1. 1+ hospitalizations with dx code for type 1 diabetes (Table S2) 2. 1+ Rx for infusion set pumps (DINs: 00905739, 00908300, 00992968, 00992976, 00992984, 0099299) 3. Identified as type 1 in DER-CA database. 4. Age < 7 at first diabetes diagnosis
Type 2 Diabetes	1. 1+ hospitalizations with diagnosis code for other diabetes or type 2 diabetes (Table S2) 2. 2+ physician visits with diagnosis code=250 and prefix=7 within 2 years 3. 1+ prescription for drugs to treat diabetes, ATC code A10 (excluding metformin) 4. 1+ prescription for metformin, ATC code A10BA, in combination with 1 physician visit with code=250 and prefix=7 5. 1+ HgA1c tests >= 6.5% in DSM data, lab code: 0653, 1146 and GLYCOSOLATED HB 6. Identified as type 2 in DER-CA database. Exclusions for incident type 2: • 1+ Rx for infusion set pumps, (see DINs above) • Age < 7 at first diagnosis • 1+ hospitalizations with diagnosis of cystic fibrosis (Table S2) • Identified as another type of diabetes in DER-CA database

Hypertension	1. 1+ hospitalizations with diagnosis code for hypertension (Table S2) 2. 1+ outpatient physician claim with diagnosis code for hypertension (Table S2) 3. 1+ prescription for anti-hypertensive drugs • diuretics, beta blocking agents, calcium channel blocking agents, agents acting on the renin-angiotensin system, atorvastatin, terazosin (See Table S4 for ATC codes) • Clonidine was excluded due to the common indication for hyperkinetic disorders of childhood.

Table 9.5 Medication prescriptions and associated Anatomical Therapeutic Chemical (ATC) codes used in hypertension diagnosis.

Drug category	ATC Codes
Antihypertensives	C02AB01/02, C02CA04, G04CA03, C02DB02, C02DC01, C02LA01, C02LB01
Diuretics	C03AA03, C03BA04, C03BA11, C03CA01, C03CA02, C03CC01, C03DA01, C03DB01, C03DB02, C03EA01
Beta Blocking Agents	C07AA02, C07AA03, C07AA05, C07AA06, C07AA12, C07AB02, C07AB03, C07AB04, C07AB07, C07AG01, C07BA05, C07BA06, C07CA03, C07CB03
Calcium Channel Blockers	C08CA01, C08CA02, C08CA04, C08CA05, C08CA06, C08DA01, C08DB01
Agents Acting on the RAS	C09AA01, C09AA02, C09AA03, C09AA04, C09AA05, C09AA06, C09AA07, C09AA08, C09AA09, C09AA10, C09BA02, C09BA03, C09BA04, C09BA06, C09BA08, C09BA10, C09CA01, C09CA02, C09CA03, C09CA04, C09CA06, C09CA07, C09CA08, C09DA01, C09DA02, C09DA03, C09DA04, C09DA06, C09DA07, C09DA08, C09DB02, C09XA02, C09XA52
Other	C10BX03, G04CA03

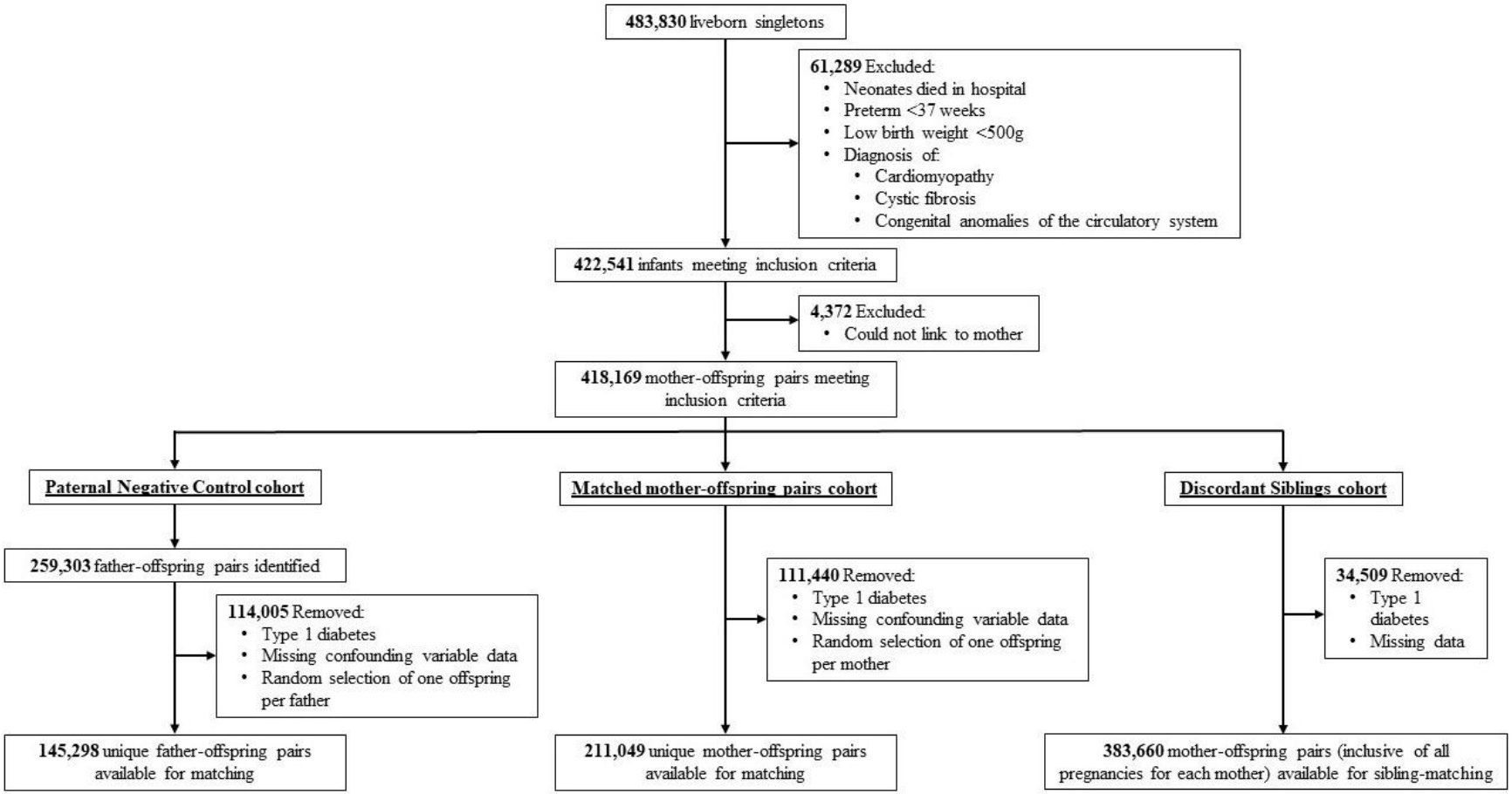

Figure 9.2 Population flow chart

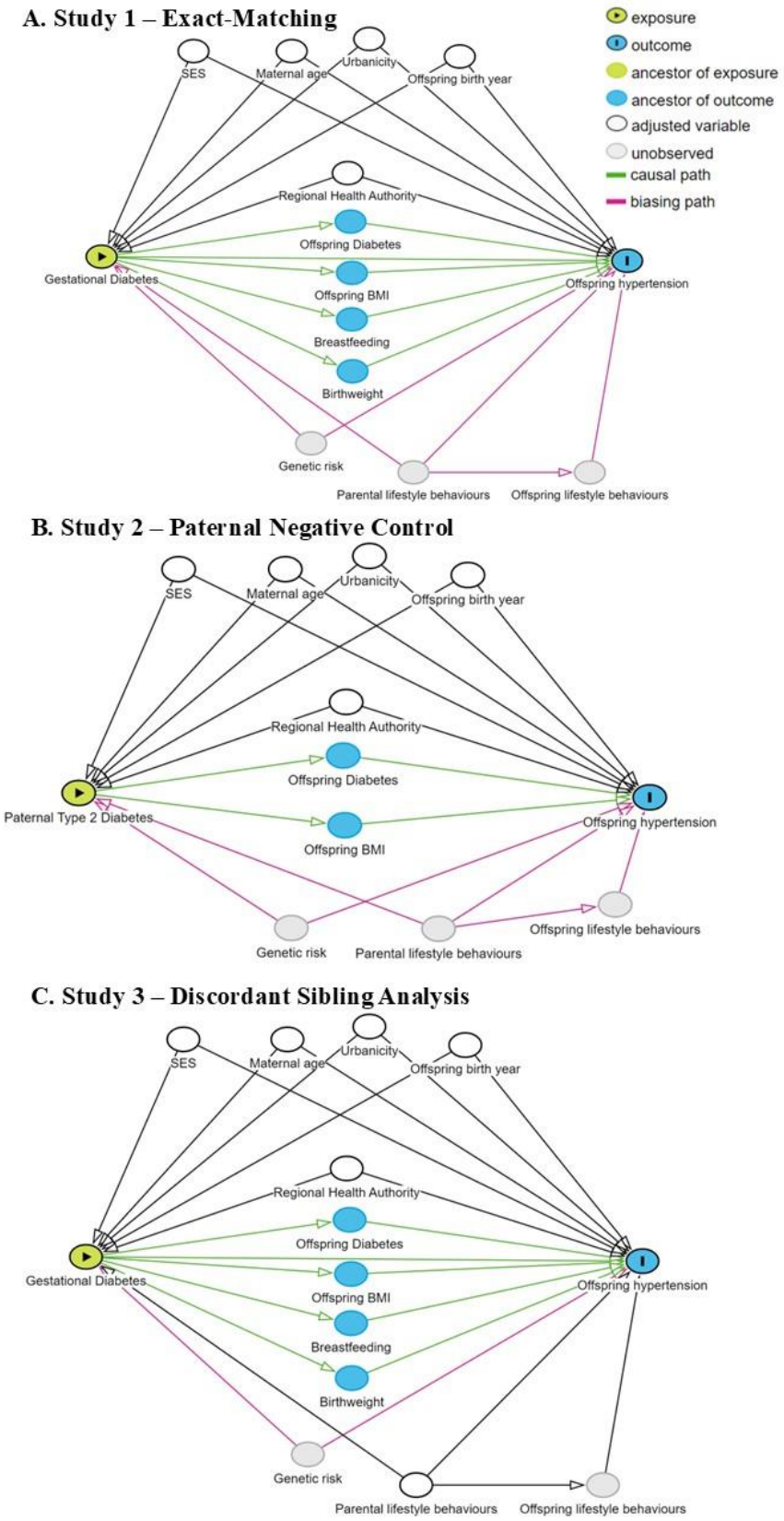

Figure 9.3. Directed acyclic graphs for each study within the triangulation study.

Table 9.6 Results from sensitivity analyses adjusting for additional variables.

Dataset	Exposure	Additional model adjustment	n	Effect	95% CI	p-value
Matched mother-offspring pairs	Gestational diabetes	Birthweight		1.40	1.15, 1.71	0.001
Matched mother-offspring pairs	Gestational diabetes	Weight for gestational age		1.39	1.14, 1.71	0.001
Matched mother-offspring pairs	Gestational diabetes	Offspring type 2 diabetes	Not enough Data			
Matched mother-offspring pairs	Gestational diabetes	Breastfeeding		1.40	1.15, 1.71	<0.001
Matched paternal-offspring pairs	Type 2 diabetes	Maternal diabetes		1.33	0.85, 2.08	0.22
Matched paternal-offspring pairs	Type 2 diabetes	Birthweight		1.48	0.95, 2.32	0.083
Matched paternal-offspring pairs	Type 2 diabetes	Weight for gestational age	Not enough data			
Matched paternal-offspring pairs	Type 2 diabetes	Offspring type 2 diabetes	Not enough data			
Matched paternal-offspring pairs	Type 2 diabetes	Breastfeeding	Not enough data			

Note: outcome for all analyses was youth-onset hypertension. Matched variables for maternal-offspring pairs were offspring birthyear, maternal age, socioeconomic index, parity, residence, and Health Region. Matched variables for paternal-offspring pairs were the same minus parity.

Table 9.7 Validity of exposure among siblings discordant for exposure to gestational diabetes

Measure	N	Mean ± SD	p-value
Fasting Blood Glucose (mmol/L)			
Exposed	51	5.3 ± 1.0	0.02
Unexposed	27	4.7 ± 0.9	
2-hour Blood Glucose (mmol/L)			
Exposed	51	8.7 ± 2.5	0.01
Unexposed	27	7.3 ± 2.1	

Delphic Chemistry Lab Tests from DSM, 2006/07-Dec 2019

Table 9.8 Assessment of confounding by indication

Prevalence of well-child physicals among offspring diagnosed with hypertension compared to those not diagnosed		
	Received physical N (%)	**p-value**
No hypertension diagnosis	97,180 (8.2)	0.13
Hypertension diagnosis	5,314 (8.1)	

Chapter 10: Study Checklists

10.1 Chapter 4 STROBE and RECORD Checklist

The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	Abstract
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction		
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction		
Methods					
Study Design	4	Present key elements of study design early in the paper	Methods - *Study Design*		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods		
Participants	6	*(a) Cohort study* - Give the		RECORD 6.1: The methods of study	Methods

		eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case		population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	Methods
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.		RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Methods - *outcomes and covariates*
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods - *outcomes and covariates*		
Bias	9	Describe any efforts to address potential sources of bias	Methods - *Analysis*		

Study size	10	Explain how the study size was arrived at	Methods - *population*		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Methods - analysis		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	Methods - *Analysis*		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population. RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	Methods - *population*
Linkage		..		RECORD 12.3: State whether the	

				study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	Methods - *study design*
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram		RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	Methods and Results (paragraph 1)
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)			Results
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or			Results

		summary measures			
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period			Results
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses			NA
Discussion					
Key results	18	Summarise key results with reference to study objectives			Discussion (paragraph 1)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias		RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	Discussion - *Limitations and conclusion*
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			*Discussion - Limitations and conclusion*

Generalisability	21	Discuss the generalisability (external validity) of the study results			*Discussion - Limitations and conclusion*
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based			NA
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	NA

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.

10.2 Chapter 5 STROBE checklist

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.