Impact of Intrauterine Exposure to Diabetes and Social Determinants of Health on Offspring Cardiovascular Disease Risk in Youth and Early Adulthood

By

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Abstract

Diabetes is a highly prevalent complication of pregnancy with rates surpassing 15% in high-risk populations. The Developmental Origins of Health and Disease theory suggests the risk for many non-communicable chronic diseases might originate *in utero*. Indeed, more than 100 000 Canadian infants are exposed to diabetes *in utero* annually and subsequently have a higher risk for type 2 diabetes and obesity early in life. These conditions correlate tightly with cardiovascular diseases and may share common origins related to intrauterine diabetes exposure. Animal experiments support this theory by reporting worse cardiometabolic markers in pups exposed to diabetes *in utero*. This relationship has not been fully explored in humans, and it is not clear if it is modifiable. This thesis attempts to address these knowledge gaps with four unique studies: 1) a cross-sectional clinical study on the effect of intrauterine diabetes exposure on left ventricular structure and function, in a cohort of youth with type 2 diabetes; 2) a prospective population-based cohort study of intrauterine diabetes exposure and cardiovascular risk; 3) a prospective population-based cohort study on the role of high school completion and breastfeeding initiation in attenuating the association between intrauterine diabetes exposure and cardiovascular risk; and 4) a systematic review and meta-analysis of trials investigating intensive management of gestational hyperglycemia to prevent childhood obesity, a major facet of cardiometabolic risk. Results show that while left ventricular structure and function measured in adolescents is not different according to intrauterine diabetes exposure, youth and young adults exposed to diabetes *in utero* are 2-3 times more likely to receive a cardiovascular disease-related diagnosis compared to those not exposed, after adjustment for potential confounders such as birth weight for gestational age and socioeconomic status. Conversely, offspring exposed to

diabetes who completed high school were 35% less likely to develop such diagnosis compared to those who did not complete high school. Breastfeeding initiation was not associated with cardiovascular risk. Finally, based on the limited existing evidence, managing hyperglycemia in pregnancy does not reduce childhood obesity. Together, these studies suggest intrauterine diabetes exposure as an independent but modifiable risk factor for cardiovascular disease in youth.

Résumé

Le diabète est une des complications de grossesse les plus prévalentes au Canada, atteignant des taux de plus de 15% chez les femmes à risque. La théorie de l'origine développementale des maladies et de la santé suggère que le risque de développer plusieurs maladies chroniques non-infectieuses pourrait apparaître pendant la vie intra-utérine. En effet, plus de 100 000 Canadiens sont exposés au diabète *in utero* et sont à risque élevé de développer du diabète de type 2 et du surpoids tôt dans leur vie. Comme ces maladies sont liées aux maladies cardiovasculaires, il est possible qu'elles partagent des origines intra-utérines. Des études animales ayant montré des marqueurs cardiométaboliques empirant après l'exposition intra-utérine au diabète supportent cette hypothèse. Cette relation n'a pas encore été complètement testée chez l'humain et il n'est pas clair si elle est modifiable. La présente thèse tente de répondre à cette question via quatre études distinctes: 1) une analyse transversale de l'effet de l'exposition intra-utérine au diabète sur la structure et la fonction du ventricule gauche chez des adolescents avec diabète de type 2; 2) une étude de cohorte prospective populationnelle du risque cardiovasculaire après l'exposition intra-utérine au diabète; 3) une étude de cohorte prospective populationnelle sur le rôle de la complétion des études secondaires et l'initiation de l'allaitement pour atténuer le risque cardiovasculaire après exposition intra-utérine au diabète; et 4) une revue systématique et méta-analyse d'essais randomisés contrôlés du traitement intensif de l'hyperglycémie de grossesse pour prévenir l'obésité infantile, une facette importante du risque cardiométabolique. Les résultats suggèrent que bien que la structure et la fonction cardiaque des adolescents avec diabète ne soit pas différentes suivant l'exposition intra-utérine au diabète, les jeunes et jeunes adultes (10 à 35 ans) exposés au diabète i*n utero* ont un risque 2 à 3 fois plus élevé de recevoir un diagnostic relié à leur santé cardiovasculaire que ceux n'ayant pas été exposé au diabète, et ce,

même après ajustement pour plusieurs facteurs de confusion potentiels comme le poids de naissance pour l'âge gestationnel et le statut socioéconomique. De même, ceux exposés au diabète mais ayant complété leurs études secondaires avaient un risque cardiovasculaire diminué de 35% compare à ceux qui étaient aussi exposés, mais n'avaient pas complété leur secondaire. Initier l'allaitement après l'accouchement n'était pas associé avec le risque cardiovasculaire dans cette étude. Finalement, selon les quelques essais trouvés dans la littérature, un traitement intensif de l'hyperglycémie de grossesse n'est pas suffisant pour prévenir l'obésité infantile. Ensemble, les études contenues dans cette thèse suggèrent aux chercheurs et cliniciens que l'exposition intra-utérine au diabète pourrait être un facteur de risque additionnel, mais potentiellement modifiable, des maladies cardiovasculaires chez les jeunes et les jeunes adultes.

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List of Abbreviations

aHR: Adjusted hazard ratio

ANOVA: Analysis of variance

BMI: Body mass index

CDC: Center for Disease Control and Prevention

CI: Confidence intervals

CVD: Cardiovascular diseases

DNA: Deoxyribonucleic acid

DOHaD: Developmental origins of health and disease theory

GDM: Gestational diabetes mellitus

iCARE: Improving renal complications of type 2 diabetes in adolescents through research cohort study.

Hb_{A1c}: Glycated hemoglobin

HR: Hazard ratio

IOTF: International Obesity Task Force

Ln: Natural logarithm

LV: Left ventricle or left ventricular

MCHP: Manitoba Center for Health Policy

NHANES: National Health and Nutritional Examination Surveys

Repository: Population Health Research Data Repository

SDoH: Social determinants of health

SEFI-2: Socioeconomic factor index version 2

T2D: Type 2 diabetes

WHO: World Health Organisation

1. Chapter 1: Introduction

1.1. Intrauterine Exposure to Diabetes

Between 6 to 10% of births in Canada are complicated by intrauterine exposure to diabetes every year and this figure rises to 15% among at-risk populations such as those with high rates of obesity or from minority ethnic groups.[1] These rates have increased in the past few decades in Canada and other developed countries and show no sign of decreasing.[2,3] The two main types of intrauterine diabetes exposures are type 2 diabetes (T2D) diagnosed before conception (henceforth called "pre-existing T2D"), and gestational diabetes mellitus (GDM) diagnosed during pregnancy, as they account for 99% of the intrauterine exposure to diabetes.[1] These two types of diabetes share risk factors (i.e., attributes that increase the likelihood of developing a disease, such as obesity, older age, familial history of diabetes[4]) and an etiology in which glucose intolerance results from an uncoupling between insulin secretory capacities and insulin resistance.[5–7] This differs from other types of diabetes such as monogenic or type 1 diabetes which have genetic and/or autoimmune origins.[8,9] Both pre-existing T2D and GDM are diagnosed through measurement of glycemia, and they are generally distinguished from each other by the timing of the diagnosis in reference to the gestational period, as GDM develops after the 20th gestational week and usually resolves within six weeks post-partum.[10] Typically, GDM is diagnosed with an oral glucose tolerance test (OGTT) undergone between 24 and 28 weeks of gestation and during which the fasting pregnant woman drinks a solution containing 75g or 100 g of glucose.[10,11] Glycemia is tested fasted and 1 h, 2 h, and/or 3 h after drinking the solution; if it exceeds a pre-specified value at any time point, GDM is diagnosed.[11] Together, the glucose concentration of the solution, the glycemic cut-offs during the OGTT, and the decision to precede the diagnostic test by a screening test constitute a GDM diagnostic criterion, and these

vary across organisations (Table 1.1). In comparison, T2D is diagnosed when fasting glycemia ≥7.0 mmol/L or 2 h glycemia during a 75 g OGTT ≥11.1 mmol/L[12] and this criterion is commonly used and accepted across organisations.[13–15] The predominance of pre-existing T2D and GDM as causes for intrauterine exposure to diabetes as well as their shared phenotypes explains why this dissertation will be focused on these two forms of diabetes. Also, because of their shared phenotype and progression patterns, they can be used to investigate dose-response, with pre-existing T2D being considered a higher dose of intrauterine diabetes exposure compared to GDM.

Table 1.1 Comparison of GDM diagnostic criterion across different organisations, from oldest to newest.

Organisation	Glucose concentration	Timing of glycemic measures	Glycemic thresholds (mmol/L)	Number of positive values needed	Preceded by a screening test?
National Diabetes Data Group, 1979[16]	100 g	Fasting 1 h 2 h 3 h	≥5.8 ≥10.6 ≥9.2 ≥8.1	2	Yes
Australian Diabetes in Pregnancy Society, 1998[17]	75 g	Fasting 2 h	≥5.5 ≥8.0	1	Yes
World Health Organisation, 1999[14]	75 g	Fasting 2 h	≥7.0 ≥7.8	1	No
International Association of Diabetes and Pregnancy Study Group, 2010[18]	75 g	Fasting 1 h 2 h	≥5.1 ≥10.0 ≥8.5	1	No
Canadian Diabetes Association, 2013[11]					
1 step	75 g	Fasting 1 h 2 h	≥5.1 ≥10.0 ≥8.5	1	No
2 steps	75 g	Fasting 1 h 2 h	≥5.3 ≥10.6 ≥8.9	1	Yes
American Diabetes Association, 2018[16]					
1 step	75 g	Fasting 1 h 2 h	≥5.1 ≥10.0 ≥8.5	1	No
2 steps	100 g	Fasting 1 h 2 h 3 h	≥5.3 ≥10.0 ≥8.6 ≥7.8	2	Yes

Intrauterine diabetes exposure is an established risk factor for metabolic diseases in the offspring. Exposure to GDM or pre-existing T2D increases the risk for T2D[19–21], obesity[20,21], and related complications[22,23] in early life and adolescence. This might be related to the fact that

pre-gestational T2D and, to a lesser extent, GDM create a fetal milieu characterised by hyperglycemia, dyslipidemia, higher inflammation, and potentially impaired adipokine signalling.[24] Fetal exposure to diabetes or to the metabolic derangements that accompany diabetes may alter the development of key fetal organs involved in cardiovascular health and wellbeing, potentially increasing children's susceptibility to chronic non-communicable diseases.[25,26] In support of this theory, animal studies and population-based natural experiments of extreme environmental exposures such as the Dutch famine found that intrauterine metabolic derangements specifically during the early to mid-pregnancy period, a period marked by rapid organogenesis, predisposes offspring to multiple cardiometabolic diseases compared to those not exposed during this period .[20,25,27–30] The idea that part of an individual's future health originates before birth has always been very common in many First Nations epistemologies, such as the Lakota[31], Six Nations[32], Cree[33], and that of many other Nations of North America.[34] Recently, this concept has been integrated into western science under the Developmental Origins of Health and Disease (DOHaD) theory following observations by western scientists[35,36], as explained in the next section.

1.2. Developmental Origins of Health and Disease Theory

The DOHaD concept, in its simplest expression, states that environmental factors acting in early life have independent and profound effects on vulnerability to diseases later in life[37] (Figure 1.1). The DOHaD theory offers a widely accepted theoretical framework to explore the posited relationship between intrauterine exposure to diabetes and offspring risk for cardiometabolic diseases.

Figure 1.1 The Developmental Origins of Health and Disease concept following intrauterine exposure to diabetes.

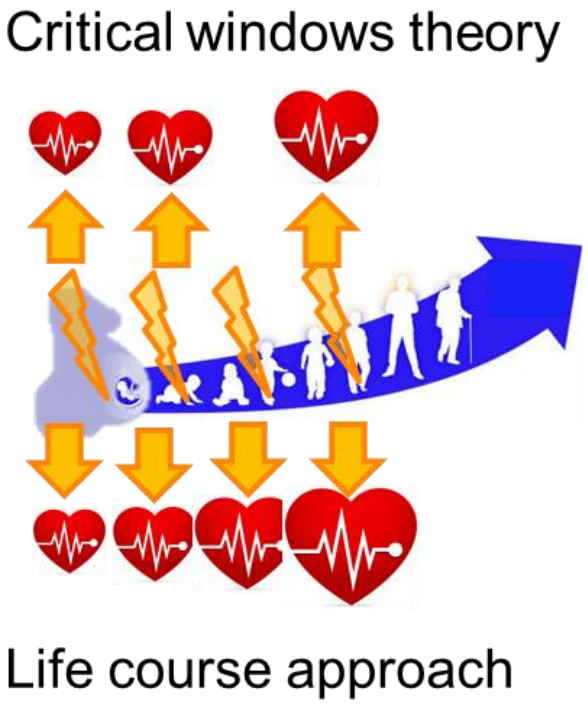
The blue "life course" arrow was used and modified with permission from the United States Developmental Origins of Health and Disease Society. This framework depicts how events that impact pregnancy, such as diabetes, might also impact the developing fetus who then grows up and experiences different stages of health and disease throughout the lifetime. GDM: gestational diabetes; T2D: pre-existing type 2 diabetes.

1.2.1. Proposed Mechanisms: Critical Windows or Life Course Approach

A proposed mechanism linking intrauterine exposures and future disease risk is the alteration of epigenomes which become uniquely sensitive to specific environmental cues during "critical windows".[38,39] Epigenetics are heritable changes in gene expression[40] or gene expression potential[41] unrelated to changes in DNA sequence. More specifically, the epigenetic responses engaged during these critical windows alter expression of genes that shape tissues and organs development – particularly during conception, gestation, the first few years of life, and puberty[42,43] – according to environmental cues and set them up for a lifetime functionality that happens to protect or predispose the individual to chronic diseases.[38,39,44,45] This concept relies on the hypothesis that epigenetic marks are the main mechanism through which environmental exposures have lasting and/or transgenerational impacts.[40,41] Indeed, certain epigenetic marks are highly stable and believed to be non-reversible (i.e., there are no known DNA demethylases to remove methylation marks on cytosine-guanine dinucleotides[46,47]). Within such constraints,

the epigenetic marks caused by intrauterine diabetes exposure and leading to increased cardiovascular diseases (CVD) risk would be maintained throughout life, but additional marks could reverse or attenuate the effects of existing DNA methylation marks during other critical windows.

Figure 1.2 Comparison of the critical windows theory and the life course approach.



The blue "life course" arrow was used and modified with permission from the United States Developmental Origins of Health and Disease Society and the other images are public domain images. The top side of the figure illustrates how an exposure, represented by the orange lightning strike, could influence cardiovascular risk, represented by the heart. In this model, only exposures occurring in the prenatal period, infancy, and puberty would impact cardiovascular risk. This is because childhood beyond the first ~2 years of life is not reputed to be a critical window. The bottom side of the figure shows how different exposures impact cardiovascular risk according to the life course approach. In this model, all four exposures impact cardiovascular risk because exposures are reputed to have potentially lasting effects irrespective of their timing.

However, this proposed mechanism is not universally accepted. Others have suggested a "life course approach" in which metabolism is always adapting to its environment and that

disease risk changes gradually, but constantly, over the entire lifetime irrespective of fixed time windows.[48,49] This approach arose from observations of the biological, psychosocial, and behavioural contexts that impact human health across a lifetime or generations and posited that various factors influence health and disease independently, cumulatively, and interactively.[50] In such a frame, even if part of someone's disease risk is inscribed during intrauterine development, exposures and interventions throughout life will counteract or amplify this risk in an incremental manner. In fact, there is preliminary evidence of the active reversibility of DNA methylation through various pathways not involving demethylases and which supports ongoing, active adaptation to environmental cues throughout life (reviewed in[51,52]). If proven to have physiological relevance, the active reversibility of DNA methylation would disrupt the currently accepted view that DNA methylation is mostly irreversible and potentially force a revision of the "critical windows" concept. Specifically in the context of this dissertation, it would suggest that epigenetic marks due to intrauterine diabetes exposure could be removed following positive environmental cues, even outside of "critical windows".[51]

Notwithstanding which theory becomes verified, we, along with others, argue that if intrauterine exposure to diabetes creates an environment that predisposes offspring to obesity and T2D[23,24,44,45,53], these adaptations might also predispose the offspring to CVD.

1.2.2. Existing Evidence that an Altered Intrauterine Environment is Associated with CVD Risk

The hypothesis guiding this dissertation and the field of DOHaD research arises from previous observations of exposures to an altered metabolic milieu and later CVD risk. In the mid-1980s, Barker and Osmond revealed that perinatal mortality in specific neighbourhoods in

the United Kingdom was associated with ischemic heart disease mortality rates 50 years later in people from the same neighbourhoods (correlation coefficient of 0.73, no p value).[36] Their seminal paper launched the theoretical framework known as the "Barker hypothesis."[36,54] These observations were corroborated using data from a historical cohort study in the Netherlands. Longitudinal follow-up of full-term offspring born between 1 November 1943 and 28 February 1947 (before, during and after the 1945 famine) in one hospital in Amsterdam and for whom detailed medical birth records were available was done 50 years later.[29,30] Those exposed to maternal famine in the first half of pregnancy exhibited three times the odds for coronary heart disease[30], 6% increased risk for glucose intolerance[29] and 7% increased obesity risk for women but not men.[55] These seminal observations led to establishing that maternal nutritional deficiencies such as protein or total calories deficiencies are associated with an increased risk of offspring hypertension, overweight, and insulin resistance in animal models.[30,56–58]

Although these epidemiological observations have linked fetal exposures to *maternal nutritional deprivation* with higher cardiovascular morbidity and mortality in adulthood[28,36,59], the longitudinal independent impact of intrauterine exposure to *overnutrition* on CVD risk has not yet been determined in humans.[60–62] Contrary to maternal nutritional deprivation which leads to a lack of nutrients to the developing fetus, intrauterine diabetes exposure leads to higher nutrient supplies (termed overnutrition). Indeed, the concentration of many essential nutrients (glucose, free fatty acids, cholesterol, amino acids, etc.) in the fetal circulation is driven by the concentration of these nutrients in the maternal circulation until the placenta is vascularized around day 21 post-conception.[63,64] The placenta then becomes an intermediate in nutrient transfer, but essential nutrients are still prioritized for the fetus.[63,64] For example, intrauterine glycemia increases linearly with maternal glycemia throughout the pregnancy, and amino acids

concentration is higher on the fetal side than on the maternal side.[63,64] Fetal overnutrition might then have disease conditioning effects similar to those seen during fetal undernutrition.[64] In fact, Barker's hypothesis which argued that intrauterine metabolic exposures influence future offspring health was built upon a body of work by Freinkel, who observed that intrauterine exposure to diabetes was strongly associated with child health and inspired the "fuel-mediated teratogenesis" hypothesis[35]. This hypothesis is supported by observations that this exposure is associated with a higher prevalence of congenital anomalies in offspring.[65] Animal models of intrauterine exposure to diabetes also support the hypothesis this exposure might increase CVD risk as they show a higher prevalence of CVD risk markers in exposed offspring.[66–68]

Robust evidence linking intrauterine diabetes exposure to offspring cardiometabolic risk comes from observational studies in high-risk groups investigating the risk of T2D in offspring following this intrauterine exposure. Early studies in Pima Indians first indicated higher prevalence of glucose intolerance, an early step on the path towards T2D, in offspring exposed to diabetes *in utero* independent of known genetic markers or the familial environment.[69,70] This fuelled case-control studies of First Nations adolescents with T2D in Manitoba which revealed a 6- to 14-fold increased risk for T2D after exposure to pre-existing diabetes and ~4-fold (95% confidence interval [CI] 2.75-6.81) increased risk for T2D after exposure to GDM.[71,72] These increased risks were recently validated in a recent longitudinal registry-based cohort study which showed a 3.03 hazard ratio (95% CI 2.44-3.76) of T2D in offspring exposed to GDM *in utero* compared to those not exposed.

Together, the data from the Dutch Famine studies linking maternal undernutrition and offspring CVD risk combined with the observations of higher offspring T2D risk following exposure to diabetes *in utero* provide a foundation for the overarching hypothesis guiding this

dissertation: intrauterine exposure to diabetes is associated with higher CVD risk in offspring. As CVD is a leading cause of morbidity and mortality worldwide, it is especially important to determine if intrauterine exposure to diabetes – in itself the most common complication of pregnancy in Canada[1,10] – is indeed a modifiable risk factor for CVD.

1.3. Cardiovascular Diseases and Early Adulthood

Cardiovascular diseases, complex conditions affecting the heart or the vascular system in a chronic and lifelong manner[73], are the most common cause of mortality due to non-communicable diseases worldwide.[74] Common CVD in Canada are ischemic heart disease, myocardial infarction, cerebral infarction, and coronary artery disease.[73,75] In 2000, CVD were the second most costly contributor to total health costs in Canada at 22.2 billion dollars.[73] In 2009, it was estimated that more than 1.6 million of Canadians were diagnosed with at least one CVD. Although adults aged 65 and older represent the highest proportion of persons hospitalized and dying from CVD[76], the incidence of CVD diagnosis rises noticeably as early as 35 years old in the general population.[73] This is concerning because at these ages (35-65 years old), people are in the prime time of their life, raising a family, building a career and actively contributing to the economy.[77] Even more concerning is that in 2007, more than two-thirds of Canadian youths aged 12 to 19 years had at least one risk factor for CVD suggesting most adolescents in Canada have an increased risk for early CVD.[73] Obesity and hypertension are major contributors specifically for early CVD – one that develops before 45 years of age.[78,79] The combination of rising rates of diabetes in pregnancy and development of modifiable CVD risk factors early in life suggests a rising burden of early CVD in future generations.

Currently, screening for CVD risk is recommended in the general population after 40 years of age.[80] Screening tools (e.g. Framingham Risk Score[81,82], Cardiovascular Life Expectancy Model[83,84], American College of Cardiology/American Heart Association score[85]) have many components related to the individual, such as medical history, renal filtration rate, blood pressure, and blood profile including glucose, lipids, and lipoproteins.[80] It follows that the main prevention and treatment strategies are aimed at the management of risk factors through lifestyle modifications and sometimes use of medication. Individuals with pre-existing conditions such as diabetes, hypertension, and obesity are recognized to be at higher risk of CVD, and thus guidelines recommend for them to be screened for CVD at any age.[80] The impact of prenatal exposures on CVD risk is still unclear. Therefore, conventional screening tools do not take them into account. If intrauterine exposure to diabetes independently raises CVD risk – and especially early CVD risk –, including this exposure in screening tools may help identify high-risk individuals and initiate interventions in early life to prevent CVD. Considering that the main cause of sudden death in <40-year-olds is attributable to undetected coronary heart disease[86–88], it is possible that these deaths could be prevented if their risk factors, which might include prenatal exposures, were better defined. Thus, clarifying if intrauterine exposure to diabetes is a risk factor for CVD is important to identify at-risk individuals not captured by the standard screening tools and to better plan the use of healthcare resources by the population in the future.

In this context, prevention and treatment strategies for CVD would benefit from expanding to a public health perspective. If new strategies could lower CVD risk for the entire population, they could have a higher impact on the incidence of future CVD than current individual-based strategies. Social determinants of health may have a potential mediating role between

intrauterine exposure to diabetes and incidence of early CVD and are particularly well suited to public health approaches, as the next section will illustrate.

1.4. Social Determinants of Health

Social determinants of health are the economic and social conditions that shape the health of individuals and communities.[89] Diabetes and CVD share a propensity to cluster with social determinants of health indicative of a lower standard of living, which hints at common social roots and risk factors between these chronic diseases. Although chronic diseases are often presented and analyzed from the biomedical standpoint – which highlights the biological reasons behind their progression and offers individualistic lifestyle options to try to control them – factors such as housing and social exclusion have also been associated with chronic diseases incidence and prognosis.[89,90] In Canada, twelve factors are recognized as social determinants of health: early life, education, employment and working conditions, food security, gender, health care services, housing, income and its distribution, social safety net, social exclusion, unemployment and employment security, and Aboriginal status[89] (Figure 1.3). Because of their established relationship with chronic diseases, these determinants, individually or collectively, might modulate the impact of intrauterine exposure to diabetes on the risk of CVD. As many of these determinants can be modified through public health policies, studying their influence on the risk of CVD following intrauterine exposure to diabetes becomes an interesting avenue to identify potential prevention and treatment strategies that can be applied to the entire population.

Figure 1.3 Social determinants of health in Canada.

Twelve social determinants of health have been identified and detailed in the Canadian context[89]. As illustrated, they affect individual health directly, but they also interact with each other to modulate health.

Five markers for specific social determinants of health were chosen for this thesis as they have previously been correlated with CVD risk and progression in older populations and they can be evaluated at the population level through the administrative registries housed at the Manitoba Center for Health Policy (MCHP).

1.4.1. Early Life Marker: Breastfeeding

Breastfeeding is a marker for the "early life" determinant of health. Breastfeeding is protective for newborns' health in many regards.[91] Breastfeeding impacts childhood blood

cholesterol[92–94], blood pressure[94,95], and blood glucose[96] positively and thus could protect against early CVD in young adults.[94,97,98] Breastfeeding also appears protective against the development of T2D in offspring whether they were exposed to GDM or not.[99] Potential benefits of breastfeeding on risk of CVD after intrauterine exposure to diabetes, particularly pre-existing T2D, are currently unknown.

1.4.2. Income and its Distribution Marker: Socioeconomic Status

Socioeconomic status has been shown to be strongly linked with cardiovascular health, with higher socioeconomic affluence correlating with improved cardiovascular outcomes.[90,100] Moreover, childhood socioeconomic status has specifically been recognized as impacting later cardiovascular health in the same way.[98] Socioeconomic affluence has a considerable impact on housing quality[101] and food security[97], two factors that may have a notable influence on diabetes and CVD risk through stress, environmental exposures, and diet quality.[97,101] Finally, this marker is also associated with breastfeeding initiation, duration, and exclusivity[99,102,103] as well as with educational attainment[104]. Thus, socioeconomic status may be an important mediator in the association between intrauterine exposure to diabetes and future risk of CVD and will be considered as a potential confounder in our analyses whenever possible.

1.4.3. Gender Marker: Sex

Gender is a social determinant of health, being a psychosocial construct of the roles, behaviours, identities and social expectations of girls, women, boys, men, and gender diverse people, whereas sex is a physiological determinant of health as it is biologically determined, is

primarily associated with sexual anatomy, and generally separates people between males and females.[105,106] There are indications that CVD appears and evolves differently in men and women.[107,108] Indeed, women might have a different risk of developing CVD and may be more at risk of dying from CVD than men[109–111], making gender a relevant factor to consider in the relationship between intrauterine exposure to diabetes and CVD risk. However, only sex is available through the administrative registries and not gender. Sex differences have also been found in the potential conditioning effects of intrauterine exposure to diabetes and in CVD risk.[67,110–112] It has been argued that there is no simple way to evaluate the separate impact of sex and gender in health studies as both factors interact together and both probably have effects on the variables of interest.[113] Sex will thus be considered a covariate while keeping in mind that some effect might be due to gender and some, to the biological factor.

1.4.4. Education Marker: High School Completion

Higher levels of education correlate with increased access to cardiovascular healthcare[114] and lower prevalence of CVD.[89,115] High school completion is a standard indicator of educational attainment in the general population as it is associated with employment and economic opportunities.[116] High school completion status is available through the administrative databases. There is currently no literature on educational attainment, including high school completion, as a protective factor towards CVD risk after intrauterine exposure to diabetes.

1.4.5. Healthcare Services Marker: GDM Diagnosis and Management

Many aspects related to healthcare services, such as accessibility, affordability, availability, and appropriateness, have important impacts on health.[117] Specifically in the context of GDM, the criterion chosen by healthcare providers to diagnose women with GDM has a large influence on the proportion of women diagnosed, from <7% to >20%.[118] The variance in criterion adopted in different healthcare settings can be explained, in part, by how these criteria were developed. Originally, they were meant to identify women at risk of developing T2D[119] whereas more recent criteria, with lower glycemic thresholds, were based on the risk of adverse perinatal outcomes in the offspring.[18,120] While GDM diagnosis followed by adequate treatment can reduce adverse outcomes in mothers and infants (reviewed in [121]), different organisations are balancing these risks differently – according to their specific populations and healthcare costs schemes – with different criteria as exposed in section 1.1.[121,122] Thus, the same pregnant woman could be diagnosed with GDM in a care center but not in another for the same glycemic values, depending on which criterion is used by the centers at that time. This leads to disparities in prenatal care and possibly in offspring CVD risk.[120,123,124] Following pressure from researchers and clinicians, many organisations have considered changing their criterion and lowering their glycemic thresholds to reduce metabolic risks in offspring.[11,60,125] There is currently no consensus on the effectiveness of lowering GDM diagnostic criterion to reduce offspring cardiometabolic risk in childhood.

1.5. Limitations to the Current State of Knowledge

Previous studies in the field of CVD prevention usually target adults because CVD incidence is known to increase mainly after 35 years of age.[73] Another important factor driving

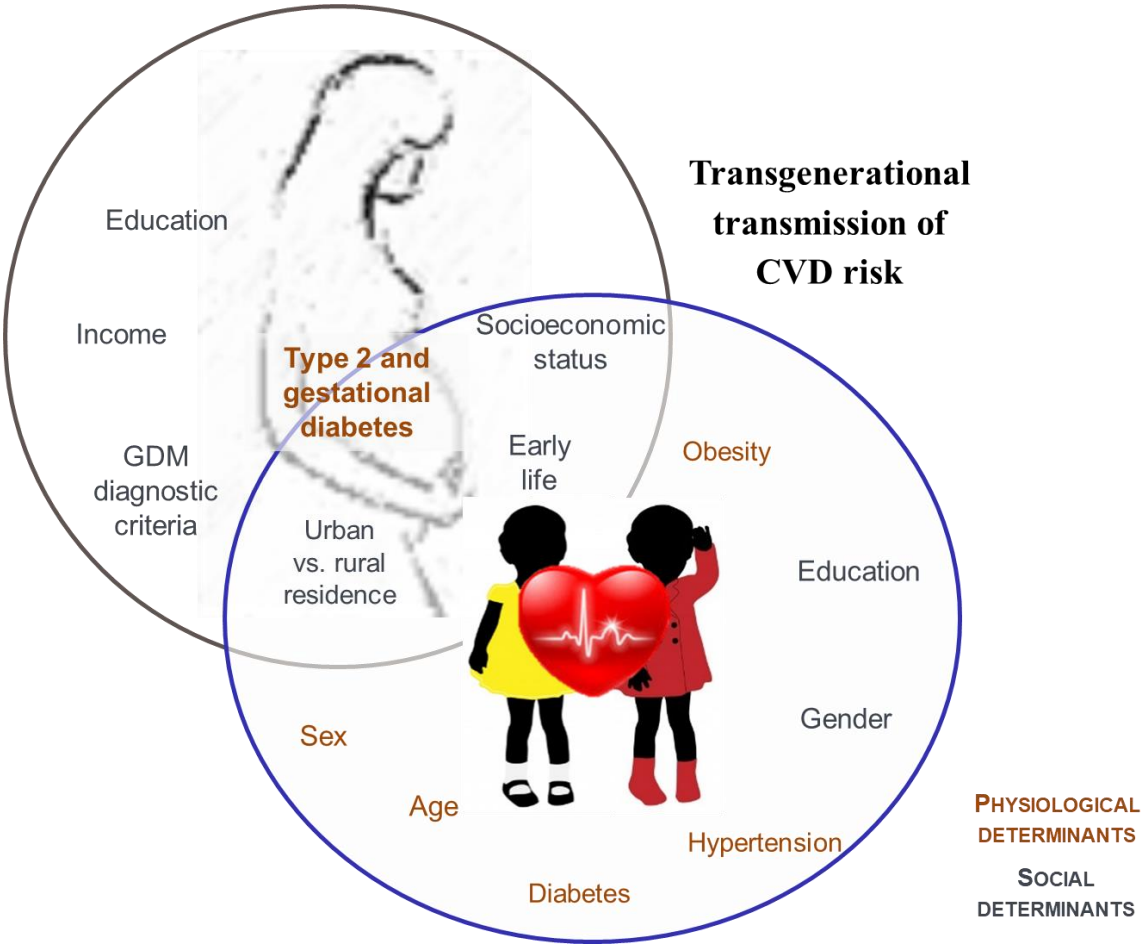
this decision is the latency between screening positive for risk factors (eg. hypertension) and truly developing CVD (eg. myocardial infarction), representing years if not decades of waiting time that is hard to manage in research.[73] Likewise, studies in the field of DOHaD are either limited to childhood outcomes, which enables good phenotyping of pregnancies and early life exposures but prevents assessment of hard CVD endpoints, or to adult outcomes, which enables the assessment of CVD endpoints but prevents a good assessment of early life exposures. This may explain why the relationship between intrauterine exposure to diabetes and CVD has not yet been firmly established in humans. It is also not known whether exposure to GDM, which develops after the critical development window (0-20 gestational weeks[126]), is comparable to or less severe than exposure to pre-gestational diabetes on the risk for CVD in humans as studies usually investigate these exposures separately. Finally, although the relationship between specific social determinants of health and CVD is well recognized in certain contexts, ~8% of trials and observational studies on CVD report any social determinants of health measure at baseline, and ~5% take them into account in results interpretation.[127] These estimates do not take into consideration studies investigating intrauterine exposure to diabetes, but we can assume such studies would be in even fewer numbers. There is a clear gap around determining if intrauterine exposure to diabetes increases the risk for CVD in humans and whether this relationship is modifiable with interventions delivered *in utero* or postnatally. Considering the high prevalence of intrauterine exposure to diabetes in Canada and Manitoba, it is relevant to study factors potentially modifiable through public policy such as social determinants of health to reduce CVD risk related to intrauterine diabetes exposure at the population level, during or after pregnancy.

2. Chapter 2. Purpose and Specific Objectives

2.1. Theoretical Framework

The theoretical framework underlying this project is inspired by both the DOHaD and the social determinants of health theories (Figure 2.1). Using these two theories, we posit that physiological and social determinants of health interact before and during pregnancy to influence the fetal milieu in a way that might protect or predispose the offspring to early CVD. Likewise, postnatal social and physiological determinants of health affecting the offspring contribute to CVD risk or protection following intrauterine exposure to diabetes or a normal environment. The advantage of this framework is that it highlights the importance of prenatal approaches for early prevention of CVD, supports multi-pronged public health initiatives, and adds consideration for modifiable social determinants in addition to traditional, biological risk factors for early CVD. An additional advantage of incorporating social determinants of health to the DOHaD model of CVD risk is the insight into potential public health policies. Whereas physiological factors are difficult to affect through policy as they rely in some part on individual choice, most social determinants of health are already governed through existing policies. These can be modified to increase favourable outcomes based on the evidence available if governments wish to design evidence-based policies.

Figure 2.1 Theoretical framework of the intergenerational transmission of CVD risk.



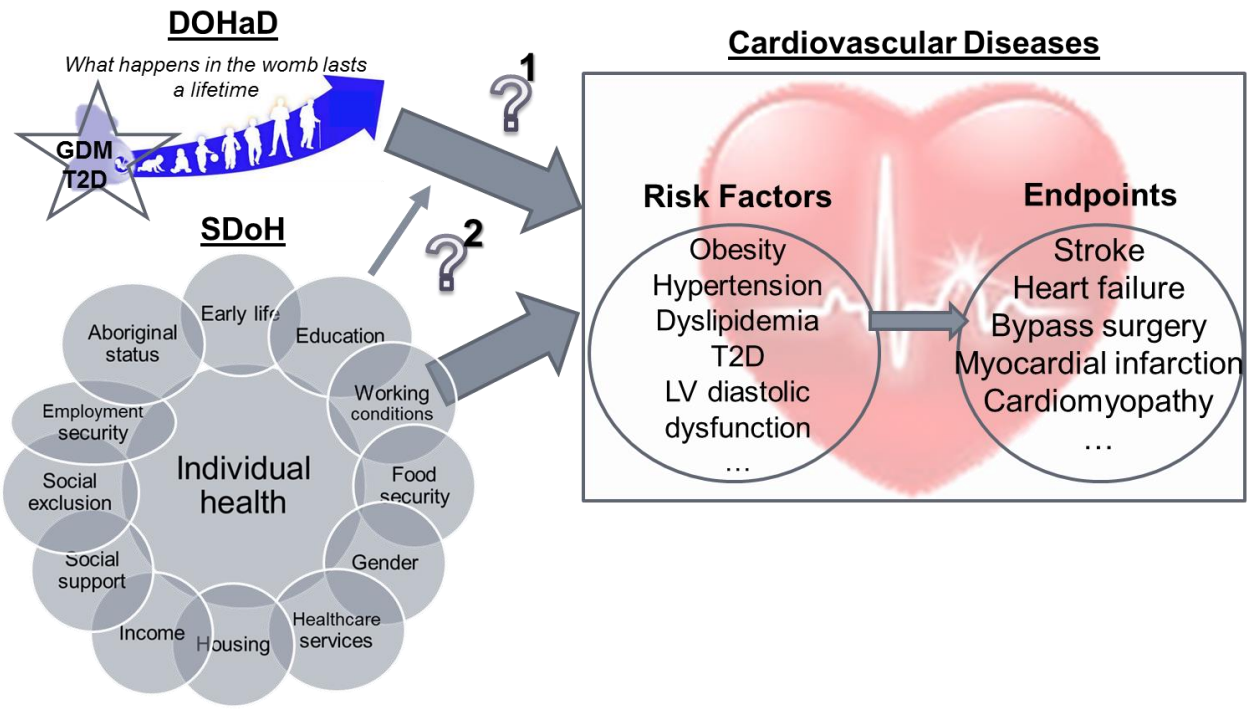
The theoretical framework on which this dissertation is founded considers the interaction between prenatal and postnatal exposures to physiological (orange font) and social (grey font) determinants of health. The upper left circle contains determinants that affect the mother's health directly before or during pregnancy and that could influence CVD risk. Similarly, the lower right circle contains modifiable and non-modifiable factors that affect the offspring directly. These factors have been correlated with CVD risk in adults and thus could also be involved in the offspring risk of early CVD. The main maternal factor of interest is intrauterine exposure to diabetes, which includes type 2 and gestational diabetes. The circles overlap because maternal factors determine part of the offspring's risk for CVD. Factors such as intrauterine exposure to diabetes, early life, socioeconomic status, and residence (urban or rural) that equally impact the mother and the offspring are situated at the intersection of both circles.

2.2. Working Hypotheses and Study Objectives

My doctoral thesis will test two working hypotheses:

1. Exposure to GDM or pre-existing T2D increases the offspring's risk for developing CVD in adolescence and early adulthood;
2. Prenatal and/or postnatal social determinants of health can modify the relationship between intrauterine exposure to diabetes and offspring CVD risk.

Figure 2.2 Overarching questions for the dissertation in an operationalized theoretical framework.



The blue "life course" arrow was used and modified with permission from the United States Developmental Origins of Health and Disease Society and the other images are public domain. This figure depicts the relationships between the main concepts discussed in this dissertation. Upper left is the DOHaD concept presented in Figure 1.1 and below it is the Social determinants of health concept from Figure 1.3. On the right is the concept of CVD, which contains cardiovascular risk factors (such as obesity, hypertension and dyslipidemia) and cardiovascular endpoints (such as myocardial infarction, heart failure and stroke). A grey relationship arrow links cardiovascular risk factors to endpoints as these risk factors are known to be related to the risk of developing CVD endpoints. A grey relationship arrow also links the Social determinants of health concept to the CVD concept to illustrate the known associations between social determinants of health and CVD risk. Two relationships in this figure are unknown; a question mark accompanies the two corresponding grey arrows. This dissertation tries to address two overarching questions: 1-Does exposure to either GDM or pre-existing T2D increases early cardiovascular risk in the offspring? 2-Can this risk be reduced through modifiable factors? The first overarching question is graphically indicated by the grey arrow going from the DOHaD concept to the cardiovascular disease concept, as indicated by the question mark #1. Likewise, the second overarching question is illustrated by the grey arrow going from the Social determinants of health concept to the grey arrow representing the posited relationship between intrauterine diabetes exposure and cardiovascular risk, as indicated by the question mark #2.

2.3. Organisation of Dissertation

This dissertation is structured using the "manuscript/sandwich style" proposed by the University of Manitoba Faculty of Graduate Studies. The dissertation contains seven chapters: the Introduction and Purpose chapters, followed by four manuscripts (chapters 3 to 6) and a summary of how the results add to the current DOHaD theory in the Discussion & conclusions chapter. Given the manuscript-based style, there is some overlap and repetition in the introduction and methods sections as well as acronym definitions in chapters 3-6. Each manuscript contains a preface explaining the contributions of the co-authors and how the manuscript fits in the dissertation. The references are continuous for the entire dissertation and can be found in a bibliography at the very end, after chapter 7. The four manuscripts presented in this dissertation were designed to test our two working hypotheses in humans. They used three distinct methodologies to balance the answers to these overarching questions according to the strengths of each design, as discussed below.

Study 1, presented in chapter 3, investigated the research question: In the context of adolescent type 2 diabetes, is intrauterine exposure to diabetes associated with differences in left ventricular (LV) function and morphology? We conducted a cross-sectional sub-study of 118 adolescents enrolled in a prospective cohort. The adolescents had their visit between November 2011 and March 2016. This study was strengthened by the use of gold standard measures including 24 h blood pressure monitoring and Doppler echocardiography.

Study 2, presented in chapter 4, addressed the research question: Is intrauterine exposure to diabetes associated with an increased risk for early CVD at the population level?

Study 3, presented in chapter 5, addressed the research question: Do postnatal social determinants of health (high school completion and breastfeeding) attenuate early CVD risk

following intrauterine exposure to diabetes at the population level? The registry-based birth cohort design used in these two studies is strengthened by the use of longitudinal data for all children born in Manitoba over the past 35 years and access to validated measures of CVD risk factors and endpoints.

Study 4, presented in chapter 6, addressed the question: Is intensive glycemic management of hyperglycemia in pregnancy effective in preventing offspring obesity? This intervention mimicked the changes in GDM diagnostic criteria proposed in many different countries in the last few years. We answered this question through a systematic review and meta-analysis. The advantages of doing so were the instant assessment of the effect of the prenatal interventions on childhood outcomes and increased statistical power compared to a single study.

3. Chapter 3. Intrauterine Diabetes Exposure and Left Ventricular Diastolic Function in Adolescents with Type 2 Diabetes

Authors: Laetitia Guillemette, Allison Dart, Brandy Wicklow, Vernon W. Dolinsky, Davinder Jassal, Elizabeth Sellers, Todd A. Duhamel, Jonathan McGavock

Manuscript status: To be submitted

3.1. Contributions of Authors

I participated in the recruitment of participants and data collection and cleaning. I was responsible for all data analysis and interpretation related to this project. I created all the figures and tables, wrote the original draft, and coordinated the revisions. I am the sole first author on this manuscript. This project was an idea of Drs. McGavock and Dolinsky, who applied for and received funding from the Heart and Stroke Foundation of Canada, and was enabled by the cohort created and followed by Drs. Dart, Wicklow and Sellers. Dr. Jassal was responsible for the interpretation of the echocardiograms.

3.2. Preface to the Manuscript

As seen in Chapters 1 and 2, considering that intrauterine exposure to T2D and GDM might increase offspring risk for cardiometabolic diseases[128,129] and that cardiometabolic risk factors (such as obesity and T2D) are more prevalent among adolescents born to mothers with diabetes compared to matched controls[19,130–133], offspring might be at risk for early LV

remodelling and dysfunction following intrauterine exposure to diabetes. The impact of this exposure on LV morphology and function in human offspring has yet to be established. The following manuscript describes the study we conducted to fill this knowledge gap.

This study addresses our working hypothesis 1: Exposure to GDM or pre-existing T2D increases the offspring's risk for developing CVD in adolescence and early adulthood. Two specific hypotheses will be tested:

1a. Exposure to pre-existing T2D leads to reduced LV diastolic function and LV remodelling indicative of cardiomyopathy in adolescents with T2D.

1b. Exposure to GDM leads to similar but less severe defects in LV filling and remodelling than exposure to T2D.

3.3. Manuscript

Abstract

Background

Type 2 diabetes (T2D) is associated with left ventricular (LV) diastolic dysfunction. It is not known if exposure to diabetes *in utero* exacerbates this dysfunction.

Methods

We performed a cross-sectional analysis of a cohort study of 118 adolescents with T2D from the iCARE cohort study who underwent echocardiography to assess if exposure to diabetes (gestational and pre-existing) *in utero* was associated with differences in LV morphology and function compared to adolescents with T2D not exposed to diabetes *in utero*. Participants' diabetes control was assessed using fasting blood glucose and glycated hemoglobin; we measured their anthropometrics (height, weight, waist circumference); and blood pressure was assessed using a 24h ambulatory blood pressure monitor. Results are presented as mean ± standard deviation or median [quartiles].

Results

Participants were 14.9 ± 2.4 years old, 65% of them were female, and 99% were Indigenous (mostly First Nations or Metis). Sixty-nine participants had been exposed to diabetes *in utero,* and they were well-matched to the participants not exposed to diabetes *in utero,* except for glycated hemoglobin which was higher in exposed participants (10.1 [7.8-11.7] % *vs* 7.9 [6.6-11.0] %, p = 0.03). Exposure to diabetes *in utero* was not associated with differences in LV

diastolic filling (not exposed to diabetes: 1.78 [1.42-2.10]; exposed to diabetes: 1.68 [1.39-2.32]) nor with differences in adjusted LV mass (74.8 ± 15.1 g/m^2 vs 80.4 ± 16.2 g/m^2, p = 0.06).

Conclusions

We did not find evidence that intrauterine exposure to diabetes is independently associated with LV diastolic dysfunction or hypertrophy in adolescents with T2D. This should be evaluated in young adults to confirm whether the absence of effect was due to the young age of the participants.

Introduction

Intrauterine exposure to diabetes, which can be divided into pre-existing type 2 diabetes (T2D) and gestational diabetes mellitus (GDM-diabetes in the last trimester of pregnancy), increases the risk for cardiovascular disease (CVD) through factors such as obesity and T2D in adolescence and young adulthood.[19–21,134] It remains unclear if this exposure independently influences the cardiovascular system of offspring. In animal studies, intrauterine exposure to diabetes is associated with direct insults to the cardiovascular system in the form of cardiac hypertrophy and/or impaired left ventricular (LV) function in fetal and early life.[66,135–143] There is a need to translate these studies into a clinical setting to determine if intrauterine diabetes exposure exerts similar effects on the cardiovascular system of youth.

Cardiovascular disease is the main cause of death and morbidity in persons living with T2D.[144,145] Echocardiographic imaging of adults with T2D displayed impaired LV filling dynamics and increased LV mass compared to controls without T2D.[146,147] Impaired LV diastolic function was also found in adolescents with T2D[148,149] and adults with conditions that precede frank diabetes (i.e. metabolic syndrome[150] and impaired glucose tolerance.[151]) These results suggest that LV diastolic dysfunction occurs before the onset of CVD. The factors that contribute to the impaired LV filling associated with T2D remain unclear but may include exposure to diabetes *in utero*. Thus, in addition to increasing the risk of T2D, intrauterine exposure to diabetes might also increase the risk for CVD by affecting LV morphology and making it vulnerable to diastolic dysfunction. We hypothesized that intrauterine exposure to diabetes would be associated with markers of LV hypertrophy and impaired LV diastolic filling in adolescents recently diagnosed with T2D. Our objectives were to identify any difference in

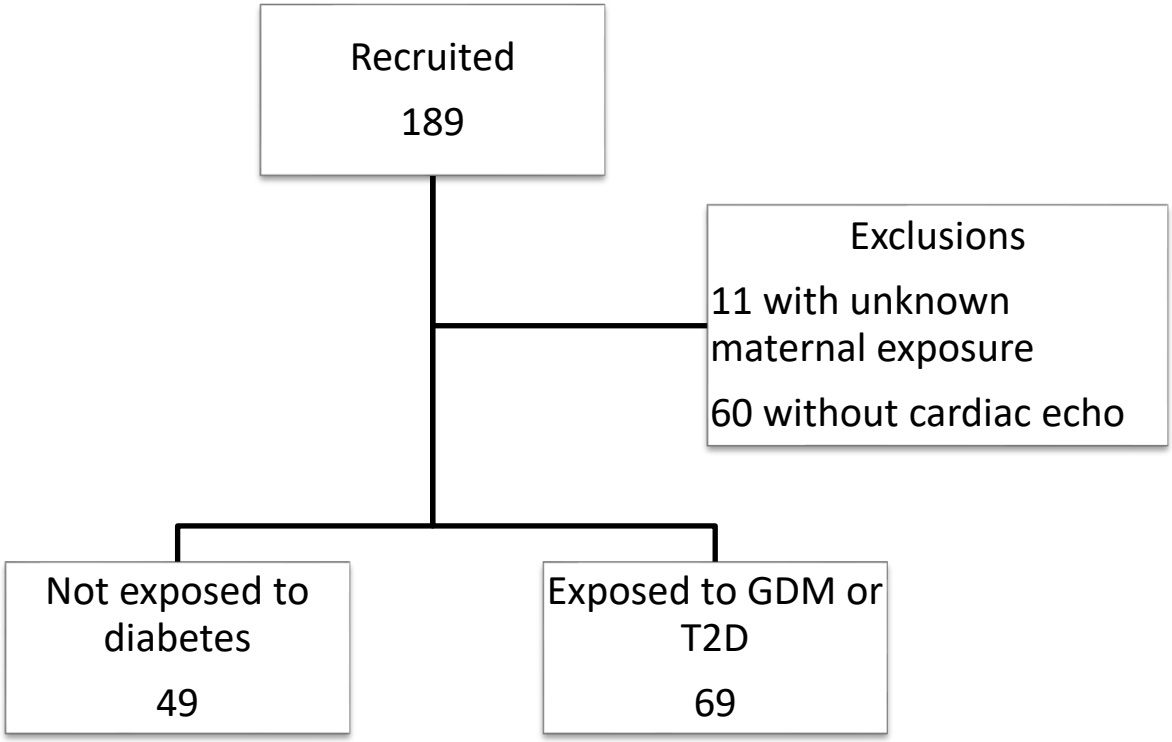
LV morphology and function between offspring exposed to diabetes *in utero* (T2D or GDM) compared to those not exposed to any diabetes *in utero*.

Research Design and Methods

Study design, setting and participants

To test the study hypothesis, we conducted a cross-sectional secondary analysis of LV structure and function among youth with T2D in the iCARE cohort study (a national expansion was registered under #NCT02818192). The STROBE guidelines for reporting observational studies were followed.[152] Adolescents with T2D were invited to participate during their routine visit at the Manitoba Diabetes Education Resource for Children and Adolescents (DERCA) clinic if they had been diagnosed with T2D (according to clinical criteria, in the absence of diabetes-related autoantibodies) and were between 10 and 25 years old.[153] Recruitment started in November 2011 and is planned to end in December 2019. Exclusion criteria were: 1) diabetes secondary to medication use or surgery; 2) antibodies suggestive of type 1 diabetes; 3) treatment with high-dose steroids or immunosuppressive agents; 4) cancer; 5) evidence of alcohol or drug abuse; 6) patient or caregiver unable or unwilling to provide voluntary informed assent/consent (as previously reported in [153]). The University of Manitoba Research Ethics Board approved the study protocol (HREB #HS13255). A parent or guardian gave written informed consent before enrollment of underaged youth in the study, and every youth gave written informed consent (if over 18 years old) or assent (if minor), in accordance with the Declaration of Helsinki.

Figure 3.1 Participant flow chart for the secondary analysis of iCARE cohort data.



GDM: Gestational diabetes mellitus. T2D: Type 2 diabetes.

Data collection

All data presented here were collected during baseline assessments, which occurred in the Children's Hospital Research Institute of Manitoba after a minimum 8 h overnight fast.

Exposure Variables

Intrauterine exposure to diabetes was divided into two categories: pooled T2D and GDM as we were underpowered to separate them, and unexposed (Control). Exposure to T2D was defined as confirmed maternal T2D diagnosis before the index pregnancy. Exposure to GDM was diagnosed when we could confirm the absence of maternal diabetes before and after the

index pregnancy in the presence of a diabetes diagnosis during the index pregnancy. Control pregnancies were assigned when the absence of diabetes before, during and after the index pregnancy could be confirmed. Eleven participants were excluded from these analyses due to our inability to ascertain their intrauterine exposure. History of exposure to diabetes *in utero* was collected in questionnaires and confirmed by a DERCA endocrinologist using medical charts.

Outcome measures

LV function The primary outcome of interest was LV filling as indicated by early-to-late mitral blood filling ratio (E/A) and early-to-late mitral annular blood flow ratio (E'/A'). Blood flow velocities were measured and gave peak (E) and late (A) LV filling, which were used to calculate E/A. Peak early (E') and late (A') velocities of mitral annular flow were acquired using tissue Doppler measured at the septal and lateral annulus and were used to calculate lateral (LAT-) E'/A' and septal (IVS-) E'/A'. Ejection fraction (%, Teicholtz formula) was used as an indicator of LV systolic function. Other functional parameters measured were deceleration time and isovolumic relaxation time.

LV structure and geometry LV internal diameter (LVID), interventricular septum thickness (IVS), and LV posterior wall thickness (LVPWT) in end diastole were used to calculate LV mass as follows: $0.8[1.04(LVID+IVS+LVPWT)^3-(LVID)^3]+0.6$, a formula validated in children.[154] LV mass was also standardized for body surface area (m^2, Haycock formula[155]) to account for height and weight differences between groups.

LV function and morphology were assessed non-invasively by one of two experienced sonographers blinded to group with a single ultrasound system (GE Healthcare Vivid, WI, USA)

following a standard protocol. All images were obtained with the participant in the left decubitus position to acquire parasternal long and short axis and apical four-chamber views. A complete M-mode two-dimensional pulsed Doppler, tissue Doppler, and colour Doppler echocardiography examination was performed on willing participants when a technician was available. Standard LV long axis, short axis, and apical imaging planes were obtained. Images were saved digitally and sent to a single experienced technician blinded to group for measurements, which were done in triplicate and averaged.

Potential Confounding

Anthropometric measurements were performed in duplicates without shoes and in light indoor clothing and were averaged for use in analyses. Body weight was measured to the nearest 0.1 kg on a calibrated digital scale and height was measured to the nearest 1.0 cm with a stadiometer (Healthometer Inc, IL, USA). Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Blood pressure was assessed using 24 h continuous ambulatory blood pressure monitoring (Spacelabs Ambulatory Blood Pressure monitors, WA, USA). Overall systolic or diastolic blood pressure over the 24-hour period was used in the analyses. A fasting blood sample was taken and sent to the Children's Hospital medical laboratory to measure fasting glucose, fasting lipids, and glycated hemoglobin (Hb_{A1c}) using assays referenced to the Diabetes Control and Complications Trial standard.[153]

Statistical analysis

All analyses were performed with SPSS Statistics 24 (IBM Analytics, NY, USA). All variables were tested for normality and ln-transformed when necessary to achieve normal distributions. Values are presented as percentages for categorical variables, as mean ± standard deviation for normally distributed variables, and as median and quartiles otherwise. Categorical variables between youth stratified according to intrauterine diabetes exposure were compared using χ^2, and continuous variables were compared using Student t-tests to detect significant differences. Pearson correlations were used to determine correlations between potential confounders and the main outcome, LV filling (E/A). Linear regression models were used to test for differences in main outcomes across intrauterine diabetes exposures adjusting for potential confounders. As recruitment is still ongoing, all analyses were considered exploratory with the 2-tailed α level for statistical significance set at 0.05 and no adjustment for multiple testing.

Results

Participants' characteristics

This study was restricted to the 118 youth with T2D who had valid echocardiography and intrauterine diabetes exposure data (mean age, 15.1 ± 2.5 years; median diabetes duration: 2.2 [1.0-4.1] years; 98.4% Indigenous, 65.6% girls; Table 3.1). Mean BMI z-score at baseline was 2.52 ± 1.0, which was elevated compared to nationally representative Canadian adolescents newly diagnosed with T2D (BMI z-scores between 1.96 and 2.21 depending on ethnicity[156]). Although the *a priori* hypothesis stated we would see differences between three levels of exposure (No diabetes, GDM, and T2D), we had to pool together offspring exposed to GDM and T2D due to limited statistical power. Baseline characteristics were not different between groups

except for height and weight which were lower in participants exposed to diabetes *in utero* compared to controls (respectively, 1.63 ± 0.09 m *vs* 1.69 ± 0.10 m, p = 0.02; and 83.3 ± 20.4 kg *vs* 91.6 ± 22.2 kg, p = 0.04) and Hb_{A1c} and prevalence of hypertension, which were higher in participants exposed to diabetes *in utero* (respectively 10.1 [7.8-11.7]% *vs* 7.9 [6.6-11.0]%, p = 0.03; and 74% *vs* 56%, p = 0.05).

Table 3.1 Baseline characteristics of iCARE participants with echocardiography data according to intrauterine diabetes exposure

Parameter	Not exposed to diabetes *in utero* (N = 49)	Exposed to GDM or T2D (N = 69)	p
Age, years	15.1 ± 2.2	14.7 ± 2.6	0.43
Ethnicity (% Indigenous)	48 (98)	68 (99)	0.81
Gender (% female)	17 (65)	45 (65)	0.99
Smoking (% yes)			
Parent/guardian	36 (74)	52 (75)	0.82
Participant	8 (16)	13 (19)	0.13
Duration of diabetes, years	2.1 [1.1-3.5]	2.3 [1.1-4.1]	0.77
Hb_{A1c}, %	7.9 [6.6-11.0]	10.1 [7.8-11.7]	0.03
Fasting glucose, mmol/L	9.1 [6.3-15.5]	11.4 [7.9-16.3]	0.08
Height, m	1.69 ± 0.10	1.63 ± 0.09	0.02
Weight, kg	91.6 ± 22.2	83.3 ± 20.4	0.04
Fat mass, %	33.5 ± 11.0	30.0 ± 9.7	0.17
BMI	32.0 ± 6.3	31.4 ± 5.7	0.13
BMI z-score	2.6 ± 1.0	2.5 ± 1.0	0.65
Total cholesterol, mmol/L	4.54 ± 1.10	4.47 ± 1.00	0.74
HDL, mmol/L	1.16 ± 0.30	1.19 ± 0.27	0.57
LDL, mmol/L	2.37 ± 0.69	2.4 ± 0.70	0.63
Triglycerides, mmol/L	1.5 [1.0-2.7]	1.6 [0.9-2.2]	0.54
24h Systolic/diastolic blood pressure, mmHg	125/72 ± 10/8	125/72 ± 9/6	0.23
Hypertension (% yes)	24 (56)	43 (74)	0.05

Results are presented as mean ± standard deviation, median [quartiles], or number (percentage). Abbreviations: BMI: Body mass index; Hb_{A1c}: Glycated hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein

Cardiac parameters according to intrauterine exposure

Comparisons of cardiac parameters are presented in Table 3.2. Interventricular septum thickness and left atrium volume were smaller after exposure to diabetes (respectively 11.26 [10.28-12.34] mm *vs* 10.36 [9.45-11.38] mm, p = 0.002; and 43.2 [35.7-49.8] mL *vs* 37.5 [29.6-45.2] mL, p = 0.02), but no functional parameters – either related to systolic or diastolic function – were statistically different between groups.

Table 3.2 Echocardiography measurements of iCARE participants according to intrauterine diabetes exposure

Parameter	Not exposed to diabetes *in utero* (n = 49)	Exposed to GDM or T2D (n = 69)	P
Cardiac structure			
LV internal diameter in end diastole, mm	45.1 ± 5.0	43.4 ± 5.1	0.08
LV posterior wall thickness, mm	9.87 [8.85-10.75]	9.20 [8.40-10.41]	0.13
Interventricular septum thickness, mm	11.26 [10.28-12.34]	10.36 [9.45-11.38]	0.002
LV mass, g	168.0 ± 42.0	146.73 ± 40.1	0.006
LV mass corrected for BSA, g/m^2	80.4 ± 16.2	74.8 ± 15.1	0.06
Left atrium volume, mL	43.2 [35.7-49.8]	37.5 [29.6-45.2]	0.02
Right atrium volume, mL	36.7 [31.3-47.3]	31.7 [23.8-41.8]	0.004
Systolic function			
LV Ejection Fraction, Teicholtz formula, %	64.0 [60.7-65.3]	63.7 [59.8-66.3]	0.77
Diastolic function			
Deceleration time, ms	196 [176-218]	215 [180-234]	0.10
Isovolumic relaxation time, ms	80.0 [67.3-92.8]	73.5 [64.3-91.0]	0.47
Peak E, m/s	1.01 ± 0.18	1.03 ± 0.21	0.48
Peak A, m/s	0.58 ± 0.14	0.60 ± 0.19	0.55
Mitral E/A	1.78 [1.42-2.10]	1.68 [1.39-2.32]	0.47
Tissue mitral LAT-E'/A'	1.70 [1.00-2.48]	1.73 [1.32-2.18]	0.69
Tissue mitral IVS-E'/A'	1.40 [1.00-1.75]	1.38 [1.00-1.66]	0.56
Heart rate (beat/min)	75 ± 11	74 ± 12	0.62

Abbreviations: BSA: body surface area; LV: left ventricular. Systolic and diastolic loads have been corrected for age, sex, and height.

Correlation between diastolic function and baseline characteristics

Correlations between LV diastolic filling velocity dynamics (E/A) and relevant clinical and biochemical parameters are presented in Table 3.3. The strongest correlation was observed between the LV E/A filling ratio and average heart rate ($r = -0.43$, $p < 0.0001$). Hb_{A1c} ($r = -0.25$,

p = 0.005) and triglycerides (r = −0.23, p = 0.01) were also significantly associated with LV E/A ratio, whereas only height (r = 0.23, p = 0.01) among anthropometrics was correlated with LV E/A ratio.

Table 3.3 Correlations between E/A and relevant baseline characteristics

Baseline variable	r	P
Age, years	0.03	0.61
Diabetes duration, years	-0.17	0.07
Height, m	**0.23**	**0.01**
Weight, kg	0.04	0.74
BMI, kg/m^2	-0.06	0.49
Body fat, %	-0.03	0.81
Total cholesterol, mmol/L	-0.17	0.07
HDL, mmol/L	0.04	0.64
LDL, mmol/L	-0.12	0.21
Triglycerides, mmol/L	**-0.23**	**0.01**
Hb_{A1c}, %	**-0.25**	**0.005**
Fasting glucose, mmol/L	-0.16	0.07
24h systolic blood pressure, mmHg	0.01	0.96
24h mean arterial pressure, mmHg	-0.11	0.27
24h average heart rate, bpm	**-0.43**	**<0.0001**

BMI: Body mass index; E/A: left ventricular diastolic filling measured at the mitral valve; Hb_{A1c}: glycated hemoglobin, HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Determinants of left ventricular diastolic function

The relationship between intrauterine diabetes exposure and diastolic function was further explored through linear regression to adjust for pre-load and post-load, known determinants of LV filling dynamics measured by Doppler[157,158], and potential confounders.[159] The fully adjusted models are presented in Table 3.4. The only variable still significantly associated with diastolic filling after adjustment was LVID (partial r = 0.32, p = 0.01), a proxy measure for LV pre-load. Sensitivity analyses using three exposure groups (controls *vs* GDM *vs* T2D) instead of

two were done but did not yield different results after adjustments (E/A and LVID: partial $r = 0.36$, $p < 0.0001$). Other sensitivity analyses included fasting glucose instead of Hb_{A1c}, height instead of weight, and tissue dynamics (E'/A') as dependent variable instead of E/A, but none gave different results (E'/A' and LVID: fully adjusted partial $r = 0.12$, $p = 0.24$; other results not shown).

Table 3.4 Linear regression models for the association E/A and relevant parameters

Parameter	Partial r	P
LV internal diameter	0.34	.001
24h diastolic blood pressure	-0.04	.68
Hb_{A1c}	-0.09	.38
Weight	-0.12	.21
Exposure group (Controls *vs* Any Diabetes)	0.10	.29

All models are mutually adjusted for the other variables in the table. E/A: left ventricular diastolic filling measured at the mitral valve; Hb_{A1c}: glycated hemoglobin; LV: left ventricular.

Discussion and Conclusion

We show that, contrary to our hypothesis, intrauterine exposure to diabetes does not explain variations in LV diastolic filling, either on its own or after adjustment for known determinants of LV diastolic filling dynamics or potential confounders. As expected, the strongest determinants for LV diastolic filling were heart rate and LVID, and this was independent of glucose control (Hb_{A1c} or fasting glucose levels), anthropometrics (height, weight), or 24 h blood pressure average (systolic or diastolic). Additionally, the markers of cardiac size that were significantly different between groups indicated smaller hearts and not hypertrophy in adolescent exposed to diabetes *in utero*.

Several groups have documented impaired LV filling and LV hypertrophy in youth with obesity and T2D, but we are the first to report echocardiography data according to intrauterine diabetes exposure. Previous studies revealed 25-48% higher LV mass and 0-17% lower LV filling in adolescents with obesity and/or diabetes compared to lean controls.[148,160,161] Several reasons might explain why we did not find LV hypertrophy or dysfunction following exposure to intrauterine diabetes exposure. First, in contrast to previous studies, the two groups we studied were very well matched on most characteristics that determine LV size and function. Second, the mean early-to-late LV filling ratios observed was 1.68 [1.39-2.32] in adolescents exposed to diabetes *in utero* compared to 1.78 [1.42-2.10] in control participants, which is lower than observed in other studies with adolescents living with or without T2D/obesity (respectively 2.13 ± 0.40[161] and 1.79 ± 0.44[148] for T2D, *vs* 2.10 ± 0.55[161] and 2.02 ± 0.53[148] for obesity). Any difference due to intrauterine diabetes exposure would have to be very large for us to reach statistical significance with our sample size. Similarly to another group who studied adolescents with T2D[162], we found that Hb_{A1c} was weakly associated with LV filling (E/A), but contrary to them BMI and blood pressure were not determinants of LV diastolic function in our study. This might be due to differences in ethnic background of participants (majority of Hispanics and non-Hispanic Blacks *vs* majority of Indigenous participants in our study), older age (18 vs 15 years old), lower average systolic blood pressure (116 *vs* 125 mmHg), or longer duration of diabetes (≥6 years *vs* ≤5 years) in their study compared to ours.

In bivariate comparisons, LVID and LV mass corrected for body surface area were nearly significant between groups ($p = 0.08$ and $p = 0.06$), which suggests that the effects of intrauterine diabetes exposure on LV diastolic function might start as an indirect, subtle effect on cardiac morphology by making the LV or the entire heart smaller. This is supported by the observation

that both right and left atria were significantly smaller in adolescents exposed to diabetes *in utero*, although statistical significance was lost when these values were adjusted for body surface area. Altogether, these smaller values might be indicative of impaired fetal development or postnatal growth and might be due to developmental or epigenetic mechanisms related to intrauterine diabetes exposure.[163,164] This might enable the hearts to function correctly at first, but be related to a reduced capacity to compensate for future demands (due to increases in adiposity or poorer metabolic health as T2D progresses, for example) and thus contribute to the early development of LV diastolic dysfunction and eventually heart failure in these persons with T2D.

The contribution of intrauterine diabetes exposure to LV diastolic filling dynamics (E/A) was explored in linear regression to adjust for known determinants of E/A (LVID, diastolic blood pressure) and potential confounders (Hb_{A1c}, fasting glucose, weight, height) that were different between our exposure groups or had previously been identified as determinants of E/A.[148,162,165] However, in our study, the only determinant that was significant in fully adjusted models was LVID. This could be explained by the small difference of LV diastolic function markers between groups or the possibility that intrauterine diabetes exposure might not contribute meaningfully to impairments in LV morphology and function in adolescents.

The strengths of this study reside in our design (prospective cohort, standard measurements), in the extensive characterisation of participants which included 24 h blood pressure monitoring, and in the careful validation of intrauterine diabetes exposure by an endocrinologist through medical charts instead of retrospective self-report by the participants or their parents. To our knowledge, this is the first study reporting echocardiography-measured cardiac parameters in adolescents according to intrauterine diabetes exposure. Limitations to this

study include the sample size and the possible lack of generalizability of our results outside of Manitoba due to our mostly Indigenous sample. Indeed, the sequelae of ongoing colonization (forced poverty, food insecurity, systemic racism, etc.) are major risk factors for diabetes[166], which put Indigenous adolescents at higher risk of both being exposed to diabetes *in utero* and of developing T2D themselves compared to adolescents of Caucasian descent or other. Added to the fact that Indigenous populations have the highest birth rate in Manitoba compared to other ethnicities[167], this explains easily why 98% of our sample is Indigenous. This is similar to the composition of the DERCA clinic (90% Indigenous patients).[156] On the other hand, the very composition of our sample makes our results very relevant for healthcare centers serving high proportions of Indigenous peoples.

In summary, we are the first to show that intrauterine exposure to diabetes does not directly promote cardiac hypertrophy or LV diastolic dysfunction in adolescents recently diagnosed with T2D. In fact, this exposure is associated with smaller left ventricles and atria, possibly decreasing the heart's capacity to adapt to future stresses. Nonetheless, it is reassuring to see that LV morphology and function is preserved in these young persons living with T2D as it reinforces the idea that there is still time to intervene to prevent the onset of CVD in this at-risk population. However, further work is necessary to determine whether intrauterine diabetes exposure does have an impact on LV morphology and function, as this might become apparent only later in adulthood.

4. Chapter 4. Intrauterine Exposure to Diabetes and Cardiovascular Disease Risk in Adolescence and Early Adulthood: A Population-Based Birth Cohort Study

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4.1. Contributions of Authors

This study was first conceived by Dr. McGavock and I. I wrote the proposal with additional input from Drs Sellers, Shen, Wicklow, Fransoo, Duhamel, Dolinsky and Gordon. I sought the necessary approvals from the MCHP, the University of Manitoba Research Ethics Board, the Health Information Privacy Committee, and the Winnipeg Regional Health Region by creating the necessary documentation (forms, proposals). I cleaned the raw data obtained from MCHP, created the birth cohort by extracting and linking the relevant variables together as well as the exposure and outcome variables (with support from the Center for Health Innovation analyst Mrs. Kristine Kroeker), completed all descriptive and inference analyses with support from MCHP analysts Mrs. Heather Prior and Yao Nie, interpreted the results with support from Drs McGavock, Duhamel, Dart, Jassal, Sellers, Wicklow, Shen, Dolinsky, and Gordon, and wrote the manuscript and created the tables and figures. I am thus the sole first author on this paper. All co-authors read the final draft of the manuscript and provided feedback based on their expertise.

4.2. Preface to the Manuscript

Intrauterine exposure to diabetes increases the prevalence of metabolic markers related to cardiovascular unhealth, such as impaired fasting glucose, elevated insulin resistance, and lipid disorders.[93,168] Studies using animal models suggest a causal role for GDM exposure in CVD risk mediated by systolic and diastolic dysfunction and LV hypertrophy.[136] However, the manuscript presented in chapter 3 reports that, contrary to results obtained using animal models, intrauterine exposure to diabetes did not seem to promote LV hypertrophy or diastolic dysfunction in a Manitoban sample of adolescents living with T2D. Current observational clinical evidence links intrauterine exposure to diabetes with biomarkers of CVD risk such as blood pressure and circulating lipids.[169,170] However, the association with established CVD risks, such as hypertension or dyslipidemia, or CVD endpoints, such as myocardial infarction or stroke, is not clear. Additionally, these studies rarely distinguish between exposure to GDM and pre-existing diabetes. Thus, the lasting impact of different types of intrauterine exposure to diabetes on the risk of developing CVD in a general, low-risk population is still unclear. The following manuscript is intended to fill this gap and answer the following question: Is intrauterine exposure to GDM or T2D an independent risk factor for CVD in early adulthood in an unselected population?

This project will address our working hypothesis 1: Exposure to GDM or pre-existing T2D increases the offspring's risk for developing CVD in adolescence and early adulthood. Two specific hypotheses will be tested:

1c. Offspring exposed to diabetes *in utero* will experience a higher incidence of CVD endpoints or CVD risk factors compared to offspring not exposed to diabetes *in utero*, independent of confounders.

1d. Offspring exposed to GDM will have an intermediate risk profile, between those not exposed and those exposed to pre-existing diabetes.

Because of the administrative nature of the data used to answer these hypotheses, it is not possible to distinguish sub-types of intrauterine diabetes exposures beyond GDM and pre-existing diabetes. Although we tried restricting exposure to pre-existing T2D, it is possible that some offspring were exposed to non-T2D, which is why the term used in chapters 4 and 5 is "pre-existing diabetes" instead of "pre-existing T2D".

4.3. Manuscript

Abstract

Importance: The role of intrauterine exposure to diabetes as a risk factor for cardiovascular disease, the primary cause of mortality worldwide, is unclear.

Objective: To determine the association between intrauterine exposure to gestational or pre-existing diabetes and subsequent cardiovascular risk in young adults.

Design, setting, participants, and methods: A registry-based birth cohort in Manitoba, Canada (1979-2005, n = 345 188), with offspring followed until March 2015. Multivariable-adjusted Cox proportional hazards modelling was used to estimate risk for the incidence of a composite cardiovascular outcome and produce adjusted hazard ratios (aHR) and 95% confidence intervals (CI) after adjustment for sex, weight for gestational age, urban residence, birth year, socioeconomic status and maternal age at delivery.

Exposures: Gestational diabetes (GDM) defined as diabetes diagnosed between 20 gestational weeks and six weeks post-partum, and pre-existing diabetes diagnosed <20 gestational weeks.

Main outcomes and measures: The primary outcome was a composite measure of incident cardiovascular disease which included: cardiac arrest, myocardial infarction, coronary artery disease, ischemic heart disease, nonrheumatic valve problems, cerebral infarction; and incident cardiovascular risk factors including hypertension, dyslipidemia, type 2 diabetes, prediabetes, atherosclerosis, cardiomyopathy, abnormal circulatory diagnostic imaging or cardiovascular function findings, diagnosed with International Classification of Disease codes and/or Anatomical Therapeutic Chemical codes. Secondary outcomes were a composite score restricted

to incident cardiovascular disease, and a composite score restricted to incident cardiovascular risk factors.

Results: The 186 939 mothers included provided data for 345 188 offspring with 3 271 933 person-years of follow-up. The mean age at latest follow up was 20.5 ± 6.4 years (range: 10-35), 48.5% of the cohort was female, and 4.1% of the cohort developed the primary composite outcome (n = 14 205), of which 1287 developed CVD and the remainder, CVD risk factors. After adjustment for confounding, the hazard for the primary outcome increased in a dose-response manner with increased duration of exposure to diabetes *in utero* (GDM: aHR 1.87, 95%CI 1.72-2.03; pre-existing diabetes: aHR 3.23, 95%CI 2.90-3.59). A similar dose-response was observed for the secondary outcomes of CVD endpoints alone (GDM: aHR 1.47, 95%CI 1.11-1.95; pre-existing diabetes: aHR 2.04, 95%CI 1.36-3.05) and CVD risk factors alone (GDM: HR 1.96, 95%CI 1.80-2.13; pre-existing diabetes: HR 3.44, 95%CI 3.09-3.83). In the fully adjusted model, male sex (aHR 0.80, 95%CI 0.78-0.83), greater maternal age at delivery (aHR 0.98, 95%CI 0.98-0.98) and urban residence (aHR 0.96, 95%CI 0.93-0.99) were associated with a lower hazard of the primary outcome.

Conclusions and relevance: In this population registry-based birth cohort, intrauterine exposure to diabetes was associated with increased risk for CVD before 35 years of age.

Introduction

Cardiovascular diseases (CVD) are the most common cause of mortality due to non-communicable diseases worldwide.[74] Although adults aged 65 and older represent the highest proportion of persons hospitalized and dying from CVD[76], the population-level incidence of CVD begins to increase as early as 35 years old.[73] The majority of adolescents in developed countries live with one or more CVD risk factors and rates of CVD continue to increase in people under 40 years of age.[73] While modifiable lifestyle factors play key roles in CVD risk, there is emerging evidence that risk may also be conferred *in utero*.

In high-income countries, diabetes is currently the most common prenatal exposure that could confer cardiovascular risk to offspring[2]. Indeed, 6 to 10% of children born in developed countries are exposed to diabetes *in utero*, either gestational diabetes (GDM) which presents in the third trimester or pre-existing diabetes which affects the pregnancy from conception[2,171]. An extensive body of clinical and pre-clinical data reveal that exposure to diabetes *in utero* is associated with adverse cardiometabolic health in childhood, specifically for markers such as insulin resistance, overweight, dyslipidemia, and blood pressure (reviewed in [172]). However, it remains unclear if this risk translates into cardiovascular morbidity in young adulthood.

Thus, we created a population-based cohort using administrative data to investigate the association between intrauterine diabetes exposure and loss of cardiovascular health in offspring age 35 years or younger. We hypothesized that intrauterine exposure to diabetes would increase the risk for CVD when compared to no exposure to diabetes *in utero*. Additionally, we hypothesized CVD risk would increase in a dose-response manner, with intrauterine exposure to pre-existing diabetes being more detrimental to cardiovascular health than exposure to GDM.

Methods

Study design

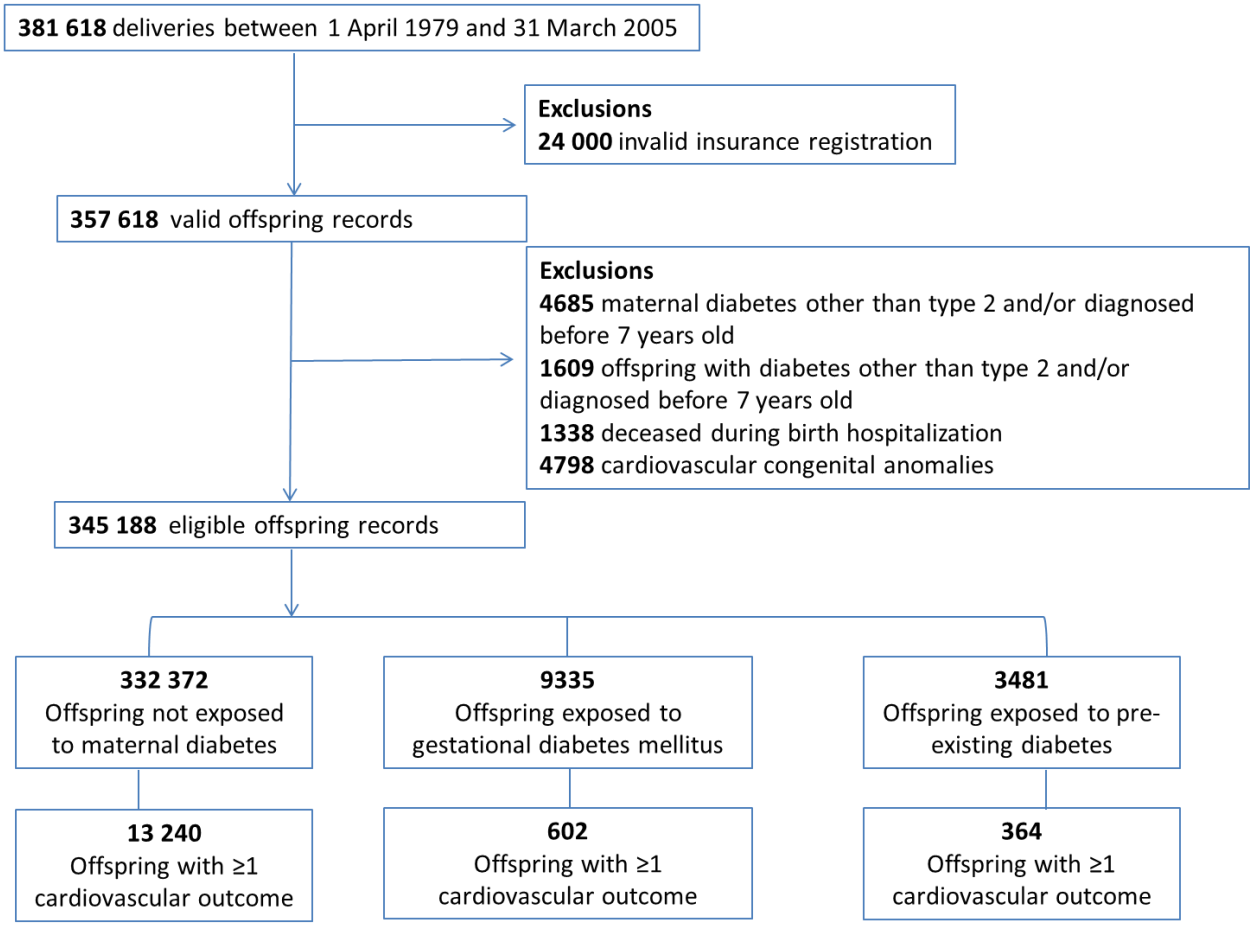
We designed an observational registry-based birth cohort study using administrative data available from 1979 in Manitoba, Canada, to test the main study hypotheses. This manuscript follows the STROBE guidelines for reporting observational studies[152] and the RECORD guidelines for studies using routinely-collected health data.[173] The Population Health Research Data Repository (the Repository) at the Manitoba Centre for Health Policy at the University of Manitoba contains linkable administrative databases including hospital discharge abstracts (1979-2015), outpatient visits (physician claims; 1979-2015), pharmaceutical prescriptions (1995-2015), the Canadian census (1979-2015), and the Diabetes Education Resource for Children and Adolescents (clinical pediatric endocrinology) database (1986-2015), for all registered individuals and uses scrambled personal health identification numbers.[174,175] The clinical pediatric endocrinology database contains pediatric endocrinology clinical care data for children <18 years of age who live with diabetes in Manitoba. This database has an ascertainment rate of >95% for type 1 diabetes and of >85% for all childhood-onset (<18 years of age) diabetes[176,177], which brings ascertainment of childhood-onset diabetes in the Repository to >95%.[177] By linking these databases, we created a unique dataset that included all children conceived and born between 1 April 1979 and 31 March 2005, followed up to 31 March 2015 (the latest date available at the time the study began, ensuring all study offspring would be at least 10 years old by the latest follow up).

Offspring were included if they could be linked to their mother and had a live birth date within the study inclusion period.[99,178] They were excluded from the cohort if they had ≥1

diagnosis of congenital anomaly of the cardiovascular system or of ineligible cardiomyopathy (endocardial fibroelastosis or congenital/familial cardiomyopathy), if they died during their birth hospitalisation, if they had invalid public health insurance coverage (as it is through this insurance number that they were tracked through databases), or if they developed non-type 2 diabetes during the study period (Figure 4.1). As administrative data cannot differentiate well between types of diabetes, all children with diabetes diagnosed at <7 years of age were excluded from the analysis because of the unlikelihood of their condition being type 2 diabetes.[177,179] In addition, children >7 years of age with type 1 diabetes in the clinical pediatric endocrinology database, prescriptions for insulin pumps, or indications of cystic fibrosis-related diabetes were excluded to further try to restrict to type 2 diabetes (total excluded n = 1609). The conditions above were excluded to limit confounding in the associations between intrauterine exposure to diabetes and CVD risk, as they are known to be associated with CVD risk but are not likely to be caused by the exposure[180–184] – except for congenital anomalies, in which case their association with intrauterine exposure is already established (reviewed in [185]).

The study was approved by the Health Research Ethics Board at the University of Manitoba (HREB #HS19742) in accordance with the Declaration of Helsinki and the provincial Health Information Privacy Committee (HIPC #2016/2017-06). Permission was obtained from the Winnipeg Regional Health Authority for the use of their clinical pediatric endocrinology database.

Figure 4.1 Flow chart for the study on the association between intrauterine diabetes exposure and CVD risk.



Exposures of interest

The primary exposure of interest was intrauterine diabetes exposure divided into categories based on the duration of exposure: GDM and pre-existing diabetes, which were used pooled and separate in the analyses. This exposure was defined from one of four datasets: hospitalisations, physician claims, clinical pediatric endocrinology, and medication prescription data as previously described.[99,179] GDM was defined as a confirmed diagnosis of diabetes (through hospital abstract forms or physician visits) between 21 weeks of gestation and six weeks post-

partum[178,186], in the absence of a non-GDM diabetes diagnosis the previous or following year. For example, two pregnancies affected by diabetes within two years, but with no additional code for diabetes in the year preceding the first pregnancy or the year following the second pregnancy were both considered to be affected by GDM. Offspring exposed to diabetes <21 weeks of gestation were considered exposed to pre-existing diabetes, and this exposure was then applied to all future siblings when applicable. Exposure to GDM and type 2 diabetes were used combined and separate in the analyses to see if effects were similar between exposures. We had enough statistical power to study each of them separately. Women with a diabetes diagnosis ≤7 years of age, a prescription for insulin pumps, or indications of cystic fibrosis-related diabetes were excluded (with their offspring) to try limit exposures to T2D and GDM (n = 15 495 mothers excluded). ICD-9-CM codes were used until 1 April 2004, and ICD-10-CA codes were used afterwards to define diabetes and GDM. These definitions of GDM and pre-existing diabetes have been used previously[178,179].

Outcomes of interest

The primary outcome of interest was the incidence of a composite cardiovascular outcome among offspring between 10 years of age to the last available follow-up period (maximum age was 35 years). The composite outcome included CVD endpoints (cardiac arrest, myocardial infarction, coronary artery disease, ischemic heart disease, nonrheumatic valve problems, cerebral infarction; ICD-9 codes 410-414, 424.0, 424.1, 424.2, 424.3, 427, 428, 429.2, 429.9, 433-435, 437.0, 437.1, 437.3, 394.0, 394.2, 394.9, 396.0; ICD 10 codes: I49, I20-I22, I24, I25, I34, I35, I46, I50, I51.9, I63, I64) and CVD risk factors (hypertension, dyslipidemia, T2D,

prediabetes, atherosclerosis, cardiomyopathy, abnormal circulatory diagnostic imaging or cardiovascular function findings; ICD-9 codes 250, 272.0-272.6, 401-404, 425.1, 425.4, 440, 459.3, 790.2, 793.99, 794.3; ICD-10 codes: E10-E14, E78.0-E78.2, E78.5, I10-I13, I70, I42.0-I42.2, I42.5, R93.1, R94.3, R73), which were ascertained through hospital abstracts, physician claims, clinical pediatric endocrinology data, and medication prescriptions (anatomical therapeutic chemical codes: A10, B01A, C01A, C01B, C01D, C02-C4, C07-C09, C10A, C10B, C01DA, R07AX01). Participants needed ≥2 visits or medical claims within three years for a diagnosis to be considered valid, ≥1 hospitalisation, and/or a diagnosis of type 2 diabetes or prediabetes in the clinical pediatric endocrinology database. This algorithm was adapted from a validated algorithm used in adults which showed moderate agreement with national survey data (kappa = 0.49), 54% sensitivity and 97% specificity[187] as well as from a validated algorithm identifying T2D in pediatric populations.[177] This modification was used because the original algorithm has not yet been validated in pediatric populations for most diagnoses used in this manuscript. Endpoints were not included in the composite outcome if they had a viral, familial, or alcoholic origin (ICD-9 codes: 424.9, 425.3, 424.5, 425.7-425.9; ICD 10 codes: E78.7, E78.8). For offspring with multiple outcomes during the follow-up period, only the first (incident) diagnosis was included, to avoid duplicates in the analysis.

Potential confounders

Offspring birth weight for gestational age was defined according to the Fenton chart[188], calculated using birth weight, sex, and gestational age at birth from the hospitalisations database. Birth weight for gestational age was then collapsed into three categories: adequate for gestational

age, small for gestational age, and large for gestational age. Preterm birth was defined as a live birth at <37 weeks of gestation.[189] Rural residence was defined as not residing in Winnipeg or Brandon (which were the urban catchment areas) as per the Canada Census data within 2.5 years of the offspring's birth. Offspring socioeconomic status at birth was determined from the SocioEconomic Factor Index 2 (SEFI-2), which is an area-based measure of socioeconomic status derived from Census data to reflect social determinants of health. It uses average household income, percent of single-parent households, unemployment rate and high school education completion rate in its calculation.[190,191] A score of 0 represents the provincial average and 95% of the scores fall within ± 2 points, with higher values indicating higher deprivation (i.e. lower socioeconomic status) and lower values indicating lower deprivation (i.e. higher socioeconomic status). Thus, each offspring was assigned the socioeconomic status of the neighbourhood in which they were born. We also adjusted for year of birth, sex, and maternal age at delivery.

Statistical methods

Descriptive statistics were used to describe the characteristics of the cohort. Analyses of variance (ANOVAs) and Chi-squares were used to investigate significant differences in baseline characteristics between groups. Cox proportional hazards regression models were used to generate adjusted hazards ratios (aHRs) and 95% confidence intervals (CI) of our composite CVD outcome in offspring as well as the adjusted survival curves after confirmation that assumptions were met. The proportional hazards assumption was tested by examining the correlation between Schoenfeld residuals and terms for time, log of time, and time squared (no

significant violations were detected) and visually checking proportionality by plotting stratified log-negative-log curves (no significant violations were detected). Individuals who died or out-migrated before reaching 10 years old or an endpoint were censored. Statistical significance was set at 0.05 using 2-sided tests, but due to the large number of observations in the dataset increasing the chance of reaching that level of significance, effect sizes of statistically significant results were discussed in addition to statistical significance to limit over-interpretation. All analyses were performed with SAS 9.3 statistical software.

Imputation

If gestational age at birth was missing, but birthweight and Apgar scores were in their normal ranges after data cleaning, a gestational age of 40 weeks was imputed (n = 73 085). No gestational age was missing after this imputation. Likewise, if gestational age and Apgar scores were within the normal range, missing birthweights were replaced by non-missing birthweight average (3448.86 g, n = 13 663). There was no missing birthweight after this imputation.

Sensitivity analyses

Models were stratified by sex as CVD literature indicates possible differences between males and females[192] and in keeping with recent calls for increased reporting of sex-stratified analyses.[193,194] Models using only the secondary composite outcomes (CVD endpoints alone, CVD risk factors alone) were also run to test the adequacy of our composite outcome compared to CVD endpoints. We also used the following approaches to evaluate if our results were

affected by confounding. Propensity scores weights based on maternal age at delivery, residence, and socioeconomic status were used and assigned based on inverse probability of intrauterine exposure to diabetes to produce adjusted estimates that would account for the unequal probability of our participants to have received one of the three exposures of interest (pre-existing diabetes, GDM, or no diabetes exposure).[195] To evaluate the impact of correlation between the participants (due to different offspring having the same mother, and thus similar genetics, households, lifestyles, etc.), we ran the final model as well as the propensity scores on a dataset restricted to the first birth of every included mother. In the full model for the primary outcome, we also replaced birth weight for gestational age with birth weight and preterm birth or used birth year categories (1979-1984; 1985-1989; 1990-1994; 1995-1999; 2000-2005) instead of continuous birth years.

Results

A total of 381 618 births from 200 754 mothers were identified in the Repository during the study inclusion period (1 April 1979 to 31 March 2005). After excluding those with invalid insurance coverage (n = 24 000), congenital anomalies (n = 4798), perinatal death (n = 1338), and invalid type of diabetes for mother and/or child (n = 6294), 345 188 offspring were included in the birth cohort (Figure 4.1). The majority were not exposed to diabetes *in utero* (96.3%), followed by those exposed to GDM (2.70%) and those exposed to pre-existing diabetes (1.01%; Table 4.1). Mean age at latest follow up (31 March 2015) was 20.5 ± 6.4 years (range: 10-35) and this was not different between exposure groups (from 21.8 ± 6.7 years in the control group to 19.5 ± 6.2 years in the pre-existing diabetes group).

Table 4.1 Characteristics of the cohort offspring (1979-2015) according to maternal diabetes exposure

	Not exposed to diabetes (n=332 372)	Exposed to GDM (n=9335)	Exposed to pre-existing diabetes (n=3481)
Age of mother at birth (years)	27.0 ± 5.5	28.5 ± 6.0	29.5 ± 5.6
Birthweight (g)	3470 ± 530	3614 ± 587	3531 ± 639
LGA	24 491 (7.4)	2140 (22.9)	968 (27.8)
SGA	27 393 (8.2)	406 (4.4)	163 (4.7)
Preterm birth	15 794 (4.8)	708 (7.6)	588 (16.9)
Female sex	163 646 (49.2)	4425 (47.4)	1701 (48.9)
Urban resident	203 248 (61.2)	5276 (56.5)	1633 (46.9)
Birth year			
1979-1984	48 786 (14.7)	484 (5.2)	148 (4.3)
1985-1989	76 802 (23.1)	2064 (22.10	708 (20.3)
1990-1994	75 443 (22.7)	2433 (26.1)	807 (23.2)
1995-1999	67 168 (20.2)	1922 (20.6)	803 (23.1)
2000-2005	64 173 (19.3)	2432 (26.1)	1015 (29.2)
SEFI-2	0.09 ± 1.10	0.50 ± 1.22	0.70 ± 1.24
Age at latest follow up (years)	21.8 ± 6.7	20.1 ± 6.2	19.5 ± 6.2

Values are mean ± standard deviation or number (%). LGA: large for gestational age; SGA: small for gestational age; preterm birth: birth <37 gestational weeks; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status).

Offspring exposed to diabetes *in utero* were more likely to have slightly older mothers (not exposed: 27.0 ± 5.5 years old; GDM: 28.5 ± 6.0 years old; pre-existing diabetes: 29.5 ± 5.6 years old; $F = 714$, $p<0.0001$), to have a large birth weight for gestational age (not exposed: 7.4%; GDM: 22.9%; pre-existing diabetes: 27.8%; $\chi^2 = 4956$, $p<0.0001$), a preterm birth (not exposed: 4.8%, GDM: 7.6%, pre-existing diabetes: 16.9%; $\chi^2 = 1216$, $p<0.0001$), and to be born in a rural area (not exposed: 38.8%; GDM: 43.5%; pre-existing diabetes: 53.1%; $\chi^2 = 370$, $p<0.0001$) in a household with a higher than average socioeconomic deprivation (not exposed: 0.09 ±1.10;

GDM: 0.50 ± 1.22; pre-existing diabetes: 0.70 ± 1.24; F = 1108, p<0.0001). Intrauterine diabetes exposure became more common over the study period, increasing from 1.3% prevalence in 1979-184 to 5.10% in 2000-2005 (χ^2 = 1271, p<0.0001).

The primary outcome was observed in 4.1% (n = 14 205) of the cohort during the follow-up period, whereas the secondary outcomes, incident CVD endpoints and CVD risk factors, were observed in 0.51% (n = 1769) and 3.8% (n = 13 136) of the cohort respectively. The three most frequent diagnoses, hypertension (n = 8712), diabetes (n = 3568) and dyslipidemia (n = 399), were responsible for 89% of the composite outcome. The crude incidence of the primary outcome rose by 1.6-fold between each exposure strata (not exposed: 3.98%; exposed to GDM: 6.45%; exposed to pre-existing diabetes: 10.4%; χ^2 = 498, p<0.0001). Most offspring experienced a CVD risk factor without any CVD endpoints (n = 12 970), and fewer reached a CVD endpoint alone without a CVD risk (n = 1287). Average age at first event was significantly earlier in exposed offspring (not exposed: 20.5 ± 6.1 years; GDM: 18.7 ± 5.9 years; pre-existing diabetes: 16.4 ± 5.3 years; F = 105, p<0.0001).

After adjusting for confounding, Cox proportional hazards regression models showed that intrauterine diabetes exposure was independently associated with risk of the composite CVD outcome (GDM vs not exposed: aHR 1.87, 95%CI 1.72, 2.03; pre-existing diabetes vs not exposed: aHR 3.23, 95%CI 2.90, 3.59, Table 4.2, Figure 4.2). Exposure to pre-existing diabetes also conferred a higher risk for the composite outcome compared to GDM exposure (aHR 1.72, 95%CI 1.51, 1.97). The strength and statistical significance of these associations remained when incident CVD endpoints and incident CVD risk factors were tested separately (Table 4.3). In the fully adjusted model for the primary composite outcome, being male and living in urban centers was protective against the composite outcome, even after adjustment (male sex: aHR: 0.80,

95%CI 0.78-0.83; urban residence: aHR 0.96, 95%CI 0.93-0.99). Likewise, being born into a lower socioeconomic status household or small or large for gestational age independently increased the risk for the composite outcome (SEFI-2: aHR 1.26, 95%CI 1.24-1.27; SGA: aHR 1.10, 95%CI 1.04-1.17; LGA: aHR 1.14, 95%CI 1.08-1.21). Finally, each consecutive year of birth and higher maternal age at delivery were statistically associated with the outcome, but the effects were small (year of birth: aHR 1.02, 95%CI 1.01-1.02; maternal age at delivery: aHR 0.98, 95%CI 0.98-0.98).

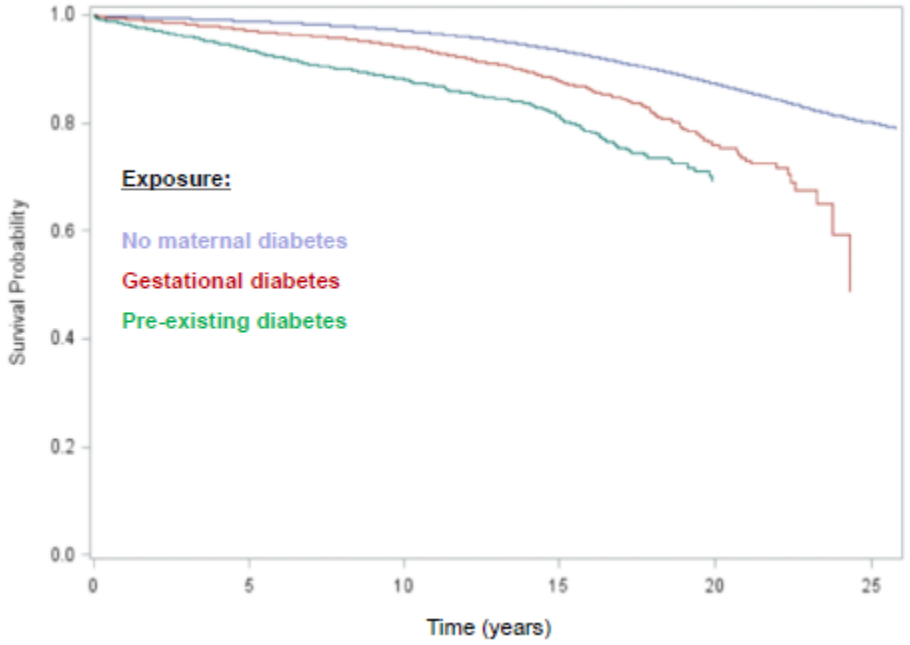
Table 4.2 Crude and mutually adjusted hazard ratios (95% confidence interval) for the primary composite cardiovascular outcome in offspring according to type of maternal diabetes exposure, compared to no maternal diabetes exposure.

	Not exposed to diabetes	Exposed to GDM	Exposed to pre-existing diabetes
Total number	332 372	9335	3481
Age at first event (years)	20.5 ± 6.1	18.7 ± 5.9	16.4 ± 5.3
Cases of the composite outcome/person-years	13 240/3 164 560	602/79 237	364/28 133
Incidence per 1000 person per year	4.2	7.6	12.9
Cases of CVD/person-years	1695/3 164 560	50/79 237	24/28 133
Incidence per 1000 person per year	0.54	0.63	0.85
Cases of cardiovascular risk factors/person-years	12 200/3 164 560	579/79 237	357/28 133
Incidence per 1000 person per year	3.9	7.3	12.7

Predictor	Crude HR (95%CI)	Adjusted HR (95%CI)
GDM	2.04 (1.88-2.21)	1.87 (1.72-2.03)
Pre-existing diabetes	3.58 (3.23-3.98)	3.23 (2.90-3.59)
Pre-existing diabetes vs GDM	1.76 (1.55-2.00)	1.72 (1.51-1.97)
Male sex	0.80 (0.78-0.83)	0.80 (0.78-0.83)
SGA	1.09 (1.03-1.15)	1.11 (1.05-1.17)
LGA	1.24 (1.17-1.31)	1.15 (1.08-1.22)
Urban residence	0.83 (0.80-0.85)	0.96 (0.93-0.99)
Birth year	1.02 (1.01-1.02)	1.02 (1.01-1.02)
SEFI-2	1.26 (1.24-1.28)	1.19 (1.17-1.21)
Maternal age at delivery	0.97 (0.97-0.97)	0.98 (0.98-0.98)

HR: Hazard ratio; CI: confidence interval; CVD: cardiovascular disease; GDM: gestational diabetes mellitus; LGA: large for gestational age; SGA: small for gestational age; preterm birth: birth <37 gestational weeks; urban: Winnipeg or Brandon; SEFI-2: measure of socioeconomic deprivation (higher index = lower socioeconomic status).

Figure 4.2 Adjusted survival curves of the risk of the primary composite outcome according to maternal diabetes exposure.



Blue: Not exposed; Red: Exposed to GDM; Green: Exposed to pre-existing diabetes. Horizontal axis is time to event or censoring, starting from 10 years old (start of "at-risk" period) until up to 15 March 2005, in years.

Table 4.3 Crude and adjusted hazard ratios (95% confidence interval) for CVD endpoints and risk factors separately, in offspring according to type of maternal diabetes exposure, compared to no maternal diabetes exposure.

Predictor	GDM (n=9335)	Pre-existing diabetes (n=3481)
CVD endpoints only	**HR (95%CI)**	**HR (95% CI)**
Maternal diabetes, crude	1.47 (1.11-1.95)	2.04 (1.36-3.05)
Maternal diabetes, adjusted for sex	1.47 (1.11-1.95)	2.04 (1.36-3.05)
Maternal diabetes, adjusted for SEFI-2	1.33 (1.00-1.76)	1.82 (1.22-2.73)
CVD risk factors only		
Maternal diabetes, fully adjusted*	1.96 (1.80-2.13)	3.44 (3.09-3.83)

SEFI-2: SocioEconomic Factor Index 2. *Adjusted for sex, birth weight for gestational age status, residence (urban vs. rural), birth year, SEFI-2, and maternal age at delivery.

Analyses stratified by sex indicated lower hazard ratios for the primary composite outcome for males compared to females, although this difference was not statistically significant either for exposure to GDM (females: HR 2.05 95%CI 1.84-2.29; males: aHR 1.67, 95%CI 1.47-1.90; Table 4.4) or pre-existing diabetes (females: aHR 3.49, 95%CI 3.49-4.03; males: aHR 2.95, 95%CI 2.51-3.46) compared to not exposed. All covariates were significantly associated with the composite outcome in the female-only model whereas only socioeconomic status (aHR 1.19, 95%CI 1.16-1.22), birth year (aHR 1.03, 95%CI 1.02-1.03) and maternal age at delivery (aHR 0.99, 95%CI 0.98-0.99) reached statistical significance in the male-only model. Pooling GDM and pre-existing diabetes exposures produced a significant hazard ratio in-between the ratios for each separate exposure (aHR 2.21, 95%CI 2.07-2.37 *vs* not exposed). Inverse probability of treatment weighting using propensity scores successfully balanced the non-exposed group and the group exposed to GDM. However, they were not able to fully balance the group exposed to pre-existing diabetes even after restricting the dataset to the first birth of every mother, so this group was excluded from the model with propensity scores. The final Cox proportional hazards model run with the propensity scores obtained similar estimates for the composite outcome (GDM compared to not exposed, aHR: 1.81, 95%CI 1.79-1.84), supporting the conclusions obtained with the original model. Original conclusions were also maintained when the dataset was restricted to the first birth of every mother (GDM aHR 1.86, 95%CI 1.66-2.09; pre-existing diabetes: aHR 2.71, 95%CI 2.27-3.23). In the model replacing birth weight for gestational age by weight and preterm birth, similar hazard ratios were obtained for intrauterine diabetes exposure compared with the original model, birth weight was not associated with the outcome (aHR 1.00, 95%CI 1.00-1.00), and preterm birth significantly increased cardiovascular risk (aHR 1.27, 95%CI 1.78-1.37). In the model using birth year categories instead of continuous birth

years, the effect sizes were maintained for intrauterine diabetes exposure and offspring born in 1995-2000 or 2000-2005 had increased risk for the outcome (respectively aHR 1.29, 95%CI 1.20-1.39; aHR 1.62, 95%CI 1.46-1.80). These last two sensitivity models resulted in decreased goodness of fit, so the original variables (birth weight for gestational age and continuous birth years) were kept for the main model.

Table 4.4 Crude and fully adjusted hazard ratios (95% confidence interval) for the composite cardiovascular outcome in offspring according to type of maternal diabetes exposure and stratified by sex, compared to no maternal diabetes exposure.

	Females		Males	
Predictor	**Crude HR (95%CI)**	**Adjusted HR (95%CI)**	**Crude HR (95%CI)**	**Adjusted HR (95%CI)**
GDM	2.23 (2.00-2.48)	2.05 (1.84-2.29)	1.83 (1.61-2.08)	1.67 (1.47-1.90)
Pre-existing diabetes	3.89 (3.38-4.47)	3.49 (3.03-4.03)	3.29 (2.81-3.85)	2.95 (2.51-3.46)
Pre-existing diabetes vs GDM	1.74 (1.46-2.07)	1.70 (1.43-2.02)	1.80 (1.47-2.19)	1.77 (1.45-2.16)
SGA	1.13 (1.04-1.22)	1.13 (1.04-1.22)	1.09 (1.00-1.18)	1.08 (1.00-1.18)
LGA	1.37 (1.27-1.48)	1.22 (1.13-1.32)	1.15 (1.06-1.25)	1.07 (0.99-1.17)
Urban residence	0.77 (0.74-0.80)	0.90 (0.86-0.94)	0.91 (0.87-0.96)	1.04 (0.99-1.10)
Birth year	1.01 (1.01-1.02)	1.01 (1.01-1.02)	1.03 (1.02-1.03)	1.03 (1.02-1.03)
SEFI-2	1.27 (1.25-1.30)	1.19 (1.17-1.22)	1.23 (1.20-1.26)	1.19 (1.16-1.22)
Maternal age at birth	0.96 (0.96-0.97)	0.97 (0.97-0.98)	0.98 (0.97-0.98)	0.99 (0.98-0.99)

Adjusted models are adjusted for all covariates in the table. GDM: gestational diabetes mellitus; LGA: large for gestational age; SGA: small for gestational age; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status).

Discussion

This population-based administrative birth cohort study reveals for the first time that intrauterine exposure to GDM or pre-existing diabetes is associated with 1.9- and 3.2-fold increased hazards for CVD within 35 years of life respectively, compared to offspring not

exposed to diabetes *in utero*. The increased CVD morbidity was driven by early-onset hypertension, type 2 diabetes, and dyslipidemia. These risks are adjusted for other known CVD risk factors such as sex, being born small or large for gestational age, and socioeconomic status, and were obtained in children free from diagnosed congenital cardiovascular anomalies. This observation supports the theory that part of CVD morbidity has intrauterine origins and extends previous findings by revealing that the risk is already evident in adolescence and early adulthood.

Fetal exposure to GDM and pre-existing diabetes has been more common over the last decades partly because of increasing rates of type 2 diabetes in younger women and partly because of improved screening and diagnosis of these conditions.[2,196] The findings of our study support and extend previous discoveries in animal models and human studies of the developmental origins of cardiometabolic diseases.[30,36,67] A causal role for intrauterine exposures in increasing CVD risk has been proposed in recent animal studies showing that maternal GDM or hyperglycemia can lead to offspring cardiac hypertrophy, systolic and diastolic dysfunction, and high blood pressure in mice and/or rats.[197,198] Small cross-sectional studies of children 5-15 years old found that exposure to GDM was associated with a higher prevalence of altered glucose metabolism, high blood pressure, and arterial stiffness[168,169,199–202] when compared to children not exposed to diabetes *in utero*. We extend these findings by demonstrating a robust dose-response association between the duration of intrauterine exposure to diabetes and both CVD endpoints and CVD risk factors. These data provide the first longitudinal population-level evidence to support the theory that CVD risk is, at least in part, programmed *in utero*.

There are known differences in how men and women are affected by CVD, with men showing a higher and earlier incidence but women showing a higher mortality rate (reviewed in [192,203]). Sex differences related to intrauterine exposures and CVD risk are also common.[204] A meta-analysis on intrauterine diabetes exposure and blood pressure in childhood showed that exposed males had higher blood pressure than non-exposed males, whereas no relationship between exposure and outcome was found in females.[169] This indicates that conditioning events might occur differently between sexes and it might be related to the known physiological differences of the cardiovascular system and the protective role of sex hormones such as estrogens in females (reviewed in [205]). In our study, male sex was significantly protective in the full model, but once the data was stratified by sex, the hazard ratios were not statistically different between males and females. Nonetheless, the ~1.2-fold higher risk in females *vs* males might be a sign of future disparities in mortality rates and is thus relevant to mention. Interestingly, significant predictors were different between the two groups, with weight for gestational age and urban residence having no significant impact on male cardiovascular health. This might point to the differential impact of the postnatal environment in the development of CVD risk, or to intrinsic differences between males and females regarding cardiovascular physiology[192,204], which we could not test with our data. In that view, our results support the use of different, more adapted measures (programs, screening tools) to prevent and treat CVD in males and females.

Limitations

A first limitation of the study is potential misclassification bias due to the use of administrative data not collected specifically for research purposes. We tried reducing it in the following manner. For our exposure, we collected diabetes/diabetes in pregnancy diagnoses from three different databases (physician claims, hospitalisations, clinical pediatric endocrinology) and attributed the exposure (gestational or pre-existing diabetes) according to the date of the diagnosis. This circumvented the problem of modified GDM criteria during the study period and of misdiagnosis of pre-existing diabetes as GDM.[179,206] However, there remains potential for women to have had GDM without being diagnosed, which would result in exposed offspring being classified as non-exposed in our data and bias our estimates towards nullity. For the composite CVD outcomes, we collected diagnoses codes in four databases (physician claims, hospitalisations, medication prescriptions, clinical pediatric endocrinology) and applied stringent definitions for confirmed diagnoses to be considered valid. However, as only 3-digit ICD-9 codes are recorded on physician claims, it is not possible to be certain that we excluded all non-type two sub-types of diabetes (including type 1 diabetes, maturity-onset diabetes of the young, etc.). Remaining bias in misdiagnosis of exposure and/or outcome would bias our estimates towards nullity. We used algorithms validated in adults, but not in pediatric populations – only the pediatric T2D definition has been validated in this population.[177] This is why we modelled our algorithm on this validated pediatric definition and amalgamated all endpoints in a composite outcome so as not to give undue weight to untested registry-based diagnoses. An additional limitation of administrative data is potential residual confounding due to variables that were not measured or not reported in the databases, which could potentially reduce the strength of our estimates if added subsequently in the models. Indeed, we had no access to clinical/lifestyle details such as blood chemistry, diet, physical activity patterns, stress management, etc. that are

known to impact both the exposure and the outcome of interest and could explain part of their relationship in our models. Likewise, conditions that were not diagnosed were not captured as part of our outcomes. It is therefore possible that offspring from any groups developed our outcomes of interest but had not been diagnosed for them yet. As a large multi-country study indicated that around 50% of adults were unaware of having hypertension before being screened by the study team[207], the actual prevalence of our composite outcome might be higher than what we reported. As it is not possible to know if the underreporting of outcome would be different between groups, it is not clear whether this would bias our estimates towards nullity or if it would artificially inflate our estimates.

Another limitation of our results is the potential failure of the assumption that our model correctly extrapolates CVD risk in offspring exposed *in utero* to pre-existing diabetes based on the data used. The failure to match this group to the two other exposures using propensity scores highlighted the possibility that this group might be fundamentally different from the other two and that we are missing important variables that could account for this difference. For example, it has been observed that in Manitoba, early-onset type 2 diabetes disproportionally affects people from Indigenous descent.[179] We could not consider this factor as we did not have access to ethnicity. Likewise, differences in lifestyle or anthropometrics could not be considered as explained previously. The consistent finding of reduced estimates for exposure to pre-existing diabetes after adjustments in our different sensitivity analyses supports the hypothesis that some unexplained confounding remains in our model for this group. Thus, although our findings were robust across all our sensitivity analyses, the increased risk reported for offspring exposed to pre-existing diabetes needs to be confirmed in future studies. Lastly, mean imputation of key missing variables (birth weight and gestational age at birth) is a limitation as it artificially

reduces variability.[208] However this simple imputation method was valid as <10% of values were missing in both cases.[208] These imputations increase the likelihood of finding significant effects related to these variables.

Conclusions

This population-level birth cohort study reveals that offspring exposed to diabetes *in utero* have a significantly increased risk of CVD within 35 years after intrauterine exposure to diabetes. Specifically, intrauterine exposure to GDM increased risk by 1.9-fold and exposure to pre-existing diabetes, by 3.2-fold compared to those not exposed to diabetes *in utero*. This stayed significant after adjusting for modifiable and non-modifiable factors at birth such as birth weight for gestational age and socioeconomic status. Risk of CVD was driven by hypertension, diabetes, and dyslipidemia which collectively accounted for 89% of the composite CVD outcome. We found that significant predictors of CVD risk were different between males and females with some indications that females might have a higher cardiovascular risk than males. This highlights the need for more reporting of sex-differences as well as potentially different, adapted clinical care for females *vs* males.

Acknowledgements

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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 Ms. Yao Nie, Mrs. Heather Prior and Mrs. Kristine Kroeker for their logistical support in cutting the required data and/or their advice for analysing it.

Supplementary table

Table 4.5 Characteristics of the cohort offspring (1979-2005) according to exposure group, stratified by sex

FEMALES	Not exposed to diabetes N=163 646	Exposed to GDM N=4425	Exposed to pre-existing diabetes N=1701
Age of mother at birth (years)	27.0 ± 5.5	28.5 ± 6.0	29.5 ± 5.6
Birthweight (g)	3410 ± 511	3568 ± 570	3483 ± 616
LGA	10 634 (6.5)	954 (21.6)	461 (27.1)
SGA	11 631 (7.1)	154 (3.5)	66 (3.9)
Preterm birth	7126 (4.4)	290 (6.6)	276 (16.2)
Urban resident	99 993 (51.1)	2495 (56.4)	821 (48.3)
Birth year			
1979-1984	25 199 (15.4)	230 (5.2)	75 (4.4)
1985-1989	37 532 (22.9)	1015 (22.9)	339 (19.9)
1990-1994	36 948 (22.6)	1140 (22.8)	398 (23.4)
1995-1999	32 675 (20.0)	889 (20.1)	375 (22.1)
2000-2005	31 292 (19.1)	1151 (26.0)	514 (30.2)
SEFI-2	0.09 ± 1.10	0.48 ± 1.23	0.71 ± 1.23
Follow up (years)	22.0 ± 6.8	20.2 ± 6.2	19.4 ± 6.3
Age at first event (years)	21.2 ± 5.7	19.1 ± 5.8	16.4 ± 5.4
Composite CVD outcome	5869 (3.5)	254 (5.2)	159 (8.9)
Total incident CVD endpoints	863 (0.53)	25 (0.56)	11 (0.65)
Total incident CVD risk factors	6860 (4.2)	338 (7.6)	203 (11.9)
MALES	**N=168 726**	**N=4910**	**N=1780**
Maternal age at delivery (years)	27.0 ± 5.5	28.6 ± 6.1	29.5 ± 6.1
Birthweight (g)	3528 ± 542	3656 ±599	3577 ± 657
LGA	13 857 (8.2)	1186 (24.2)	507 (28.5)
SGA	15 762 (9.3)	252 (5.1)	97 (5.5)
Preterm birth	8668 (5.1)	418 (8.5)	312 (17.5)
Urban resident	103 255 (61.2)	2781 (56.6)	812 (45.6)
Birth year			
1979-1984	23 587 (14.0)	254 (5.2)	73 (4.1)
1985-1989	39 270 (23.3)	1049 (21.4)	369 (20.7)
1990-1994	38 495 (22.8)	1293 (26.3)	409 (23.0)
1995-1999	34 493 (20.4)	1033 (21.0)	428 (24.0)
2000-2005	32 881 (19.5)	1281 (26.1)	501 (28.2)

SEFI-2	0.09 ± 1.10	0.50 ± 1.21	0.69 ± 1.25
Follow up (years)	21.7 ± 6.7	20.0 ± 6.2	19.5 ± 6.2
Age at first event (years)	19.6 ± 6.4	18.0 ± 6.0	16.3 ± 5.2
Composite CVD outcome	7371 (4.5)	348 (7.9)	205 (12.1)
Total incident CVD endpoints	832 (0.49)	25 (0.51)	13 (0.73)
Total incident CVD risk factors	5340 (3.2)	241 (4.9)	154 (8.7)

Values are mean ± standard deviation or number (%). LGA: large for gestational age; SGA: small for gestational age; preterm birth: birth <37 gestational weeks; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status); CVD: cardiovascular disease.

5. Chapter 5. Social Determinants of Health Protecting against Cardiovascular Disease Risk among Offspring Exposed to Diabetes *In Utero*: a Registry-Based Birth Cohort Study.

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Manuscript status: To be submitted

5.1. Contributions of Authors

This study was first conceived by me and Dr. McGavock. I wrote the proposal with additional input from Drs Sellers, Shen, Wicklow, Fransoo, Duhamel, Dolinsky and Gordon. I sought the necessary approvals from the MCHP, the University of Manitoba Research Ethics Board, the Health Information Privacy Committee, and the Winnipeg Regional Health Region by creating the necessary documentation (forms, proposals). I cleaned the raw data obtained from MCHP, created the birth cohort by extracting and linking the relevant variables together as well as the exposure and outcome variables (with support from the Center for Health Innovation analyst Mrs. Kristine Kroeker), completed all descriptive and inference analyses with support from MCHP analysts Mrs. Heather Prior and Yao Nie, interpreted the results with support from Drs McGavock, Duhamel, Dart, Jassal, Sellers, Wicklow, Shen, Dolinsky and Gordon, and wrote the manuscript and created the tables and figures. I am thus the sole first author on this paper. All co-authors read the final draft of the manuscript and provided feedback based on their expertise.

5.2. Preface to the Manuscript

As presented in section 1.4, biological factors are not the only factors that can modify the risk for cardiometabolic diseases, and in fact, non-biological factors are easier to target through public health policies.[209,210] Some factors associated with social determinants of health, such as breastfeeding, education, and socioeconomic status, have been shown to be associated with CVD risk in general populations.[89,97,98,100,115] However, no studies were identified that investigated if these factors could be leveraged to reduce the CVD risk that is attributable to intrauterine exposure to diabetes. Accordingly, I took advantage of the cohort described in chapter 4 to evaluate if breastfeeding initiation and high school completion, factors to which children are exposed after their intrauterine exposure to GDM or T2D, were protective towards cardiometabolic risk while taking into account socioeconomic status, sex (as no information on gender is available through the Repository), and other potential confounders. The question the following manuscript answers is: "Are breastfeeding initiation and high school completion associated with a lower CVD risk in offspring exposed to GDM or T2D?". Our main interest was offspring exposed to diabetes *in utero*, but we also wanted to compare our results with existing literature which is focused on offspring not exposed to diabetes *in utero*. This is why we report results on both sub-groups (exposed and non-exposed) but focus mainly on diabetes-exposed offspring. Repetitive methods from Chapter 4 have been described in the appendix to Chapter 5.

This project will address our working hypothesis 2: Prenatal and/or postnatal social determinants of health can modify the relationship between intrauterine exposure to diabetes and offspring CVD risk. Two specific hypotheses will be tested:

2a. Our chosen markers of social determinants of health will modulate the risk for CVD after intrauterine exposure to diabetes.

2b. Our chosen markers of social determinants of health will have additive effects on the risk for CVD after intrauterine exposure to diabetes.

5.3. Manuscript

Abstract

Importance: Intrauterine exposure to diabetes increases the risk for cardiovascular disease (CVD) during adolescence and early adulthood. It is not yet known whether postnatal social determinants of health can modify this risk.

Objective: To determine if postnatal modifiable factors such as breastfeeding initiation and high school completion can reduce the risk of CVD in offspring exposed to diabetes *in utero*.

Design, setting, participants, and methods: A registry-based birth cohort of offspring stratified between exposed or not to diabetes *in utero* in Manitoba, Canada (1988-2005, n = 165 201), followed until March 2015. Multivariable-adjusted Cox proportional hazards modeling was used to estimate risk for the incidence of a composite cardiovascular outcome and produce adjusted hazard ratios (aHR) and 95% confidence intervals (CI) after adjustment for sex, birth weight for gestational age, residence (urban vs. rural), birth year, socioeconomic status, and maternal age at delivery.

Exposures: Main exposures of interest were breastfeeding initiation after birth and high school completion.

Main outcomes and measures: The main outcome measure was a composite measure of incident CVD endpoints which included: cardiac arrest, myocardial infarction, coronary artery disease, ischemic heart disease, nonrheumatic valve problems, cerebral infarction; and incident CVD risk factors including hypertension, dyslipidemia, type 2 diabetes, prediabetes, atherosclerosis, cardiomyopathy, abnormal circulatory diagnostic imaging or cardiovascular

function findings, diagnosed with International Classification of Disease codes and/or Anatomical Therapeutic Chemical codes.

Results: Within the Repository, 6651 offspring exposed to diabetes *in utero* provided 68 894 person-years follow-up. Mean age at follow up was 22.2 ± 3.1 years (range: 17-28), 47.1% of the cohort was female, and 517 (7.8%) offspring developed the composite outcome. After adjustment for confounding, the hazard ratio for breastfeeding was not significant (aHR 0.91, 95%CI 0.69-1.20) whereas high school completion was significantly protective against CVD risk (aHR 0.65, 95%CI 0.51-0.82). Similar effects were observed among offspring not exposed to diabetes *in utero* (n = 158 550; 1 601 583 person-years follow up; breastfeeding initiation aHR 1.02, 95%CI 0.91-1.14; high school completion aHR 0.58, 95%CI 0.54-0.63). In the fully adjusted model for offspring exposed to diabetes *in utero*, male sex and urban residence were significantly protective, whereas large birthweight for gestational age, year of birth and lower socioeconomic status were associated with an increased risk for CVD.

Conclusions and relevance: In this registry-based birth cohort study, breastfeeding initiation did not protect offspring exposed to diabetes *in utero* from CVD, but completing high school conferred significant protection between 17 and 28 years of age irrespective of intrauterine exposure to diabetes.

Introduction

Every year in Canada, 6 to 10% of infants are exposed to diabetes in the womb[171]. This intrauterine exposure to diabetes increases their risk of dyslipidemia, inflammation, type 2 diabetes, and high blood pressure in childhood and adolescence.[168,169] The most commonly tested approach to attenuate this risk is to normalize maternal glycemia during pregnancy[211–213]. Less commonly, studies that have explored the role of postnatal factors were generally focused on lifestyles changes in the offspring to reduce CVD risk and have not specifically targeted people exposed to diabetes *in utero* (reviewed in [214]). To date, few studies have explored social factors that could attenuate cardiometabolic risk following intrauterine exposure to diabetes.

As CVD remains the leading cause of mortality worldwide[74], social determinants of health are interesting levers to prevent and reduce CVD incidence in high-risk populations. Social determinants of health are the economic and social conditions that shape the health of individuals and communities[89] and can be modified through public health policy to influence a large number of individuals at the same time – as opposed to lifestyle interventions which only affect the individuals targeted.[215–217] The social factors that protect offspring from early-onset CVD, particularly following intrauterine exposure to diabetes, remain poorly understood.

This study aimed to examine the impact of breastfeeding initiation and high school completion[94,97,98], two key social determinants of health associated with intrauterine exposure to diabetes and adult CVD, on early cardiovascular health. Our hypothesis was that breastfeeding initiation and high school completion would individually and synergistically reduce CVD risk compared to those not breastfed or without a high school diploma, particularly among offspring exposed to diabetes *in utero*.

Method

Study design, setting, and participants

This manuscript follows the STROBE guidelines for reporting observational studies[152] and the RECORD guidelines for studies using routinely-collected health data.[173] We designed an observational registry-based birth cohort study using administrative data for all pregnancies in the province available from 1979 in Manitoba, Canada, to test the main study hypotheses. The Population Health Research Data Repository (the Repository) at the Manitoba Centre for Health Policy at the University of Manitoba contains linkable administrative databases, including physician claims, hospital discharge abstracts (1979-2015), outpatient visits (physician claims; 1979-2015), pharmaceutical prescriptions (1995-2015), the Canadian census (1979-2015), the Diabetes Education Resource for Children and Adolescents (clinical pediatric endocrinology) database (1986-2015), and the Enrollment, Marks, and Assessments database (1996-2015) for all registered individuals and uses scrambled personal health identification numbers.[174,175] The clinical pediatric endocrinology database contains pediatric clinical care data for children <18 years of age with diabetes in Manitoba. This database has an ascertainment rate of >95% for type 1 diabetes and of >85% for all childhood-onset (<18 years of age) diabetes[176,177], which brings ascertainment of childhood-onset diabetes in the Repository to >95%.[177] By linking these databases, we created a unique data set that included all children conceived and born between 1 April 1979 and 31 March 2005, followed up to 31 March 2015 (the latest date available at the time the study began).

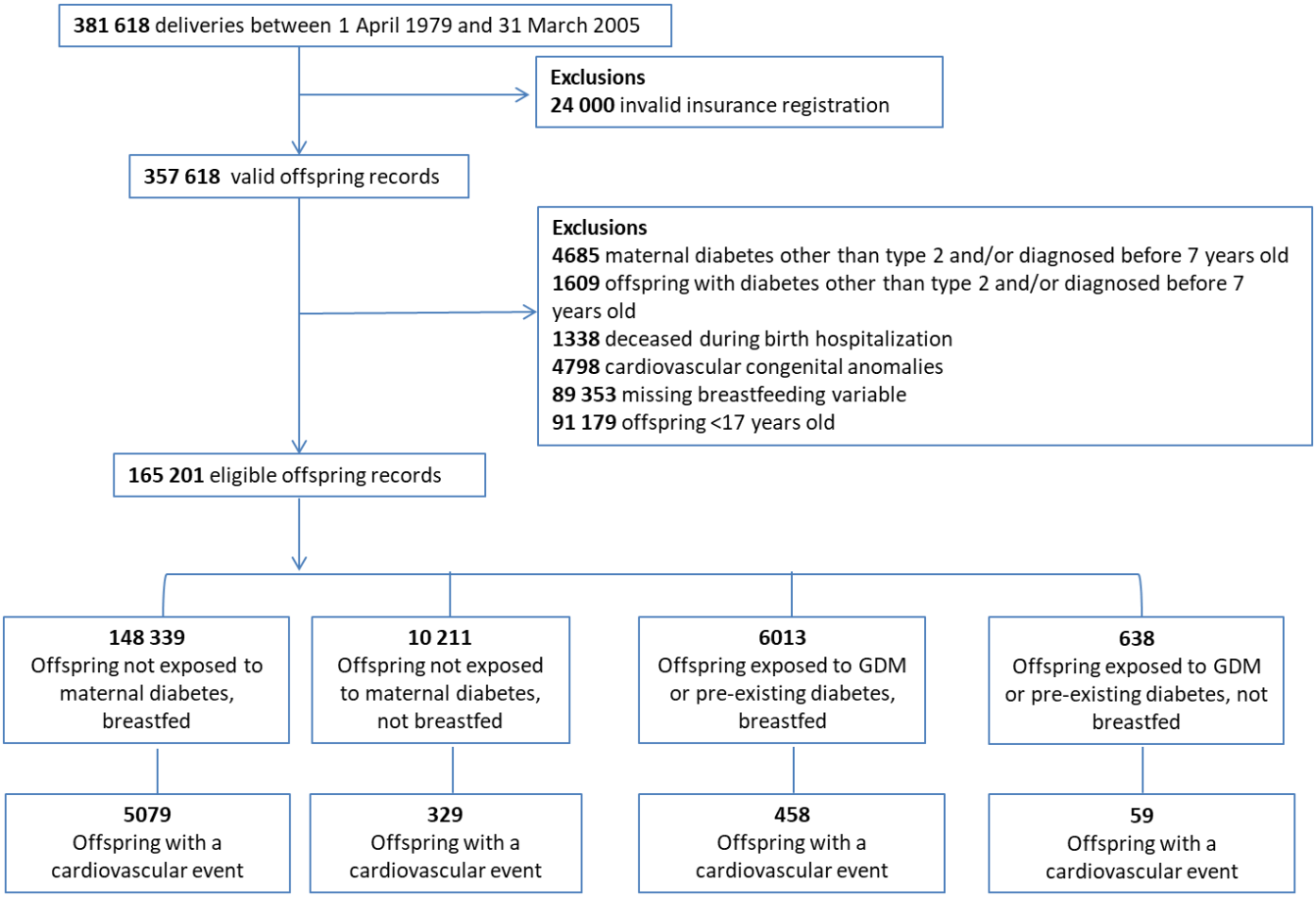
Offspring were included if they could be linked to their mother and had a live birth in a hospital within the study inclusion period (as in [99,178]). In Manitoba, 99% of all births happen in a hospital setting.[218] Offspring were excluded from the cohort if they had ≥1 diagnosis of

congenital anomaly of the cardiovascular system or of uneligible cardiomyopathy (endocardial fibroelastosis or congenital/familial cardiomyopathy), if they died during their birth hospitalisation, if they had invalid public health insurance coverage (as it is through this insurance number that they were tracked through databases), or if they developed type 1 diabetes during the study period (Figure 5.1). As administrative claims data cannot accurately differentiate between type 1 diabetes and type 2 diabetes diagnoses, all offspring with diabetes diagnosed at <7 years of age were excluded from the analysis because of the unlikelihood of their condition being type 2 diabetes. In addition, offspring >7 years of age with type 1 diabetes in the clinical pediatric endocrinology database, prescriptions for insulin pumps, or indications of cystic fibrosis-related diabetes were excluded to further try to restrict to type 2 diabetes (total excluded n = 1609). The dataset was then restricted to those who had a valid breastfeeding exposure (n = 255 835) and were at least 17 years old by the latest follow up (n = 254 009), as that is typically the earliest age at which Manitobans would complete high school. Intrauterine diabetes exposure consisted of GDM and pre-existing diabetes (mostly T2D) exposure. These exposures were pooled as we were interested in the effect of breastfeeding and high school completion following exposure to any type of diabetes and because we were underpowered to study them separately. These exposures were defined from one of four datasets: hospitalisations, physician claims, clinical pediatric endocrinology, and medication prescription data. GDM was defined as a confirmed diagnosis of diabetes (through hospital abstract forms or physician visits) between 21 weeks of gestation and six weeks post-partum.[178,186] Offspring exposed to diabetes <20 weeks of gestation were considered exposed to pre-existing diabetes. Women with a diabetes diagnosis ≤7 years of age, a prescription for insulin pumps, or indications of cystic fibrosis-related diabetes were excluded (with their offspring) to try limit exposures to T2D and

GDM. ICD-9-CM codes were used until 1 April 2004, and ICD-10-CA codes were used afterwards to define diabetes and GDM. These definitions of GDM and pre-existing diabetes have been used previously[99,179] and preliminary validation using hospital charts indicate registry-based diagnoses are ~90% accurate.

The study was approved by the Health Research Ethics Board at the University of Manitoba (HREB #HS19742) in accordance with the Declaration of Helsinki and the provincial Health Information Privacy Committee (HIPC #2016/2017-06). Permission was also obtained from the Winnipeg Regional Health Authority and Manitoba Education and Training for the use of their databases.

Figure 5.1 Flowchart of the study on the association between social determinants of health and CVD risk after intrauterine exposure to diabetes.



GDM: Gestational diabetes mellitus.

Exposures of interest

The first exposure of interest was breastfeeding initiation. This information became available on hospital abstract forms in 1987 and was recorded by nurses before hospital discharge after delivery. There is currently no information on breastfeeding duration in the Repository, but previous literature shows that initiation information is 76% accurate at least eight weeks post-partum.[219] The second exposure of interest was high school completion. High

school completion was obtained from the Enrollment, Marks, and Assessments database and represents students who completed Grade 12 of schooling, including those who completed it as mature students[220].

Outcomes of interest

The primary outcome of interest was the incidence of a composite outcome of incident CVD-related events among offspring between 10 years of age and the last available follow-up period (maximum age was 28 years). The composite outcome included incident CVD endpoints (cardiac arrest, myocardial infarction, coronary artery disease, ischemic heart disease, nonrheumatic valve problems, cerebral infarction) and incident CVD risk factors (hypertension, dyslipidemia, T2D, prediabetes, atherosclerosis, cardiomyopathy, abnormal circulatory diagnostic imaging or cardiovascular function findings; see Supplementary table 5.7). These were ascertained through hospital abstracts, physician claims, clinical pediatric endocrinology data, and medication prescriptions (anatomical therapeutic chemical codes: A10, B01A, C01A, C01B, C01D, C02-C4, C07-C09, C10A, C10B, C01DA, R07AX01). To be conservative, as some diagnoses have not yet been validated in pediatric populations in the Repository, offspring needed ≥2 visits or medication claims within 3 years for a diagnosis to be considered valid and the latest of the two dates was considered the official diagnosis date, ≥1 hospitalisation, and/or a diagnosis of T2D or prediabetes in the clinical pediatric endocrinology database. This algorithm was adapted from a validated algorithm used in adults which showed moderate agreement with national survey data (kappa = 0.49), 54% sensitivity and 97% specificity[187] as well as from a validated algorithm identifying T2D in pediatric populations.[177] Endpoints were not included in the composite outcome if they had a viral, familial, or alcoholic origin. For offspring with

multiple outcomes during the follow-up period, only the first (incident) diagnosis was included, to avoid duplicates in the analysis.

Potential confounders

Offspring socioeconomic status at birth was determined from the SocioEconomic Factor Index 2 (SEFI-2). This index is an area-based measure of socioeconomic status derived from Census data to reflect social determinants of health and uses average household income, percent of single-parent households, unemployment rate and high school education completion rate in its calculation.[191] A score of 0 represents the provincial average and 95% of the scores fall within ± 2 points, with higher values indicating higher deprivation (i.e. lower socioeconomic status) and lower values indicating lower deprivation (i.e. higher socioeconomic status). Offspring weight for gestational age was defined according to the Fenton chart[188], calculated using birth weight, sex, and gestational age from the hospitalisations database, and then collapsed into three categories: adequate for gestational age, small for gestational age, and large for gestational age. Preterm birth was defined as a live birth at <37 weeks gestation[189] and was used in lieu of birth weight for gestational age in sensitivity analyses. Rural residence was defined as not residing in Winnipeg or Brandon (which were the urban catchment areas) as per the Canada Census data within 2.5 years of the offspring's birth. We also adjusted for year of birth, sex, and maternal age at delivery.

Imputation

If gestational age at birth was missing but birthweight and Apgar scores were in their normal ranges after data cleaning, a gestational age of 40 weeks was imputed (n = 73 085). No gestational age was missing after this imputation. Likewise, if gestational age and Apgar scores were within the normal range, missing birthweights were replaced by non-missing birthweight average (3448.86 g, n = 13 663). There was no missing birthweight after this imputation.

Statistical methods

All analyses were stratified between offspring exposed and not exposed to diabetes *in utero* as the main interest was offspring exposed to diabetes *in utero*, but we also wanted to compare our results with existing literature which is focused on non-exposed offspring. Descriptive statistics were used to describe the baseline characteristics of the cohort and Student t-tests and Chi-square tests were used to investigate significant differences between groups according to breastfeeding status. Unadjusted risks were calculated using incidence rates. Multivariable-adjusted Cox proportional hazards regression models were used to test for an association between intrauterine diabetes exposure and the composite outcome of interest, and to generate adjusted hazards ratios (aHRs) and 95% confidence intervals (CI) as well as adjusted survival curves after confirmation that assumptions were met. The proportional hazards assumption was tested by examining the correlation between Schoenfeld residuals and terms for time, log of time, and time squared (no significant violations were detected) and visually checking proportionality by plotting stratified log-negative-log curves (no significant violations were detected). Models were adjusted for the potential confounders enumerated above: birth weight for gestational age, sex, socioeconomic status, maternal age at birth, birth year, and

residency (urban vs. rural). Individuals who died or out-migrated before reaching an endpoint were censored. Statistical significance was set at 0.05 using 2-sided tests, but due to the large number of observations in the dataset increasing the chance of reaching that level of significance, special attention was put on the effect sizes of statistically significant results to limit over-interpretation. All analyses were performed with SAS 9.3 (SAS Institute, Cary, USA).

Sensitivity analyses

We used tests of interaction to examine the synergy between breastfeeding initiation and high school completion, and between sex and high school completion. Models were also sex-stratified as CVD literature indicates possible differences between males and females in CVD risk[192,204] and in keeping with recent calls for increased reporting of sex-stratified analyses.[193,194] We also ran the models on a dataset restricted to complete cases for high school completion to evaluate the effect of missing high school values. In the full model for the primary outcome, we also used birthweight (continuous) and gestational age at birth (preterm *vs* term) instead of birth weight for gestational age, and birth year categories (1985-1989; 1990-1994; 1995-1999) instead of continuous birth years.

Results

A total of 381 618 deliveries from 200 754 mothers were identified in the Repository during the study inclusion period (1 April 1979 to 31 March 2005). We excluded offspring with invalid insurance coverage (n = 24 000), congenital anomalies (n = 4798), perinatal death

(n = 1338), invalid type of diabetes for mother and/or child (n = 6294), and those who had not reached their 17th birthday at latest follow up (n = 91 179). Additionally, we excluded 89 353 offspring without valid breastfeeding initiation information as it only became available in 1985 and was not captured in 26% of discharge charts. Following these exclusions, 165 201 offspring remained in the final birth cohort (Figure 5.1).

Among offspring exposed to diabetes *in utero* (n = 6651, 68 894 person-years), the majority (72.8%) were exposed to GDM, most (78.3%) were breastfed at hospital discharge, and most (73.8%) completed high school (Table 5.1). Average age at latest follow up was 21.2 ± 3.1 years (range: 17-28), and these were not different between sub-groups stratified according to breastfeeding initiation. Compared to offspring that did not initiate breastfeeding in-hospital, those who did were less likely to have been born preterm (16.7% *vs* 9.4%, $\chi^2 = 37.4$, $p<0.0001$), and into social deprivation (average SEFI-2: 0.61 ± 1.27 *vs* 0.43 ± 1.22, $p = 0.0005$). They were also more likely to be female (43.0% *vs* 47.6%, $\chi^2 = 4.97$, $p = 0.03$), born before 1994 (62.8% *vs* 77.1%, $\chi^2 = 63.9$, $p<0.0001$), have an adequate birth weight for gestational age (62.9% *vs* 71.6%, $\chi^2 = 21.6$, $p<0.0001$), and to live in an urban center (47.3% *vs* 60.0%, $\chi^2 = 36.2$, $p<0.0001$).

Table 5.1 Characteristics of cohort offspring (1986-2005) exposed or not to maternal diabetes, according to breastfeeding status at hospital discharge.

	Exposed to maternal diabetes		Not exposed to maternal diabetes	
	Breastfed (n = 6013)	Not breastfed (n = 638)	Breastfed (n = 148 339)	Not breastfed (n = 10 211)
Maternal age at birth (years)	28.7 ± 3.0	29.0 ± 6.2	27.0 ± 5.4	26.8 ± 5.7
Birthweight (g)	3582 ± 598	3583 ± 791	3469 ± 527	3381 ± 706
LGA	1421 (23.6)	195 (30.6)	12 702 (8.6)	1025 (10.0)
SGA	286 (4.8)	42 (6.6)	10 421 (7.0)	1032 (10.1)
Preterm birth	565 (9.4)	109 (17.1)	6279 (4.2)	1283 (12.6)
Female sex	2861 (47.6)	274 (43.0)	72 695 (49.0)	4984 (48.8)
High school completed*	3117 (74.0)	279 (71.5)	93 719 (83.5)	5746 (79.9)
Urban resident	3589 (60.0)	302 (47.3)	97 631 (65.8)	5059 (49.5)
Birth year				
1985-1989	1732 (28.8)	159 (24.9)	42 647 (28.8)	2201 (21.6)
1990-1994	2901 (48.3)	242 (37.9)	69 639 (24.3)	4081 (40.0)
1995-1999	1380 (23.0)	237 (37.2)	36 053 (24.3)	3929 (38.5)
SEFI-2	0.43 ± 1.22	0.61 ± 1.27	0.04 ± 1.11	0.23 ± 1.14
Age at latest follow up (years)	22.3 ± 3.0	21.5 ± 3.4	22.2 ± 3.1	21.3 ± 3.22

Values are mean ± standard deviation or number (%). LGA: large for gestational age; SGA: small for gestational age; preterm birth: birth <37 gestational weeks; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status). *Missing among exposed: n = 2050; among non-exposed: n = 39 162

Breastfeeding initiation and cardiovascular health in offspring

The primary composite outcome occurred in 517 (7.8%) diabetes-exposed offspring and 5408 (3.3%) non-exposed offspring. Among offspring exposed to diabetes *in utero*, the most frequent diagnoses, diabetes (n = 271) and hypertension (n = 193), were responsible for 89.7% of the composite outcome. The incidence of the composite outcome was not significantly different in offspring who were breastfed at hospital discharge compared to those not breastfed (7.3% *vs* 9.7% respectively; $\chi^2 = 2.14$, p = 0.14, Table 5.2). Average age at first event was later in

breastfed offspring compared to those that did not initiate breastfeeding (17.3 ± 1.2 *vs* 15.7 ± 4.2 years; p = 0.008).

Table 5.2 Crude and fully adjusted hazard ratios (95% confidence interval) for the composite cardiovascular outcome in offspring according to maternal diabetes exposure.

	Exposed to maternal diabetes		**Not exposed to maternal diabetes**	
	Breastfed	**Not breastfed**	**Breastfed**	**Not breastfed**
Total number	**6013**	**638**	**148 339**	**10 211**
Age at first event (years)	17.3 ± 1.2	15.7 ± 4.2	17.8 ± 4.5	17.6 ± 4.6
Cases/person-years	458/62 782	59/6112	5079/1 504 120	329/97 463
Cases per 1000 person-year	7.3	9.7	3.4	3.4
Crude HR	0.74 (0.56-0.96)	Reference	0.95 (0.85-1.07)	Reference
Predictor	**Crude HR (95%CI)**	**Adjusted HR (95%CI)**	**Crude HR (95%CI)**	**Adjusted HR (95%CI)**
Breastfeeding	0.74 (0.56-0.96)	0.91 (0.69-1.20)	0.95 (0.85-1.07)	1.02 (0.91-1.14)
High school completion	0.47 (0.38-0.59)	0.65 (0.51-0.82)	0.54 (0.51-0.58)	0.58 (0.54-0.63)

Adjusted for missing high school completion values.

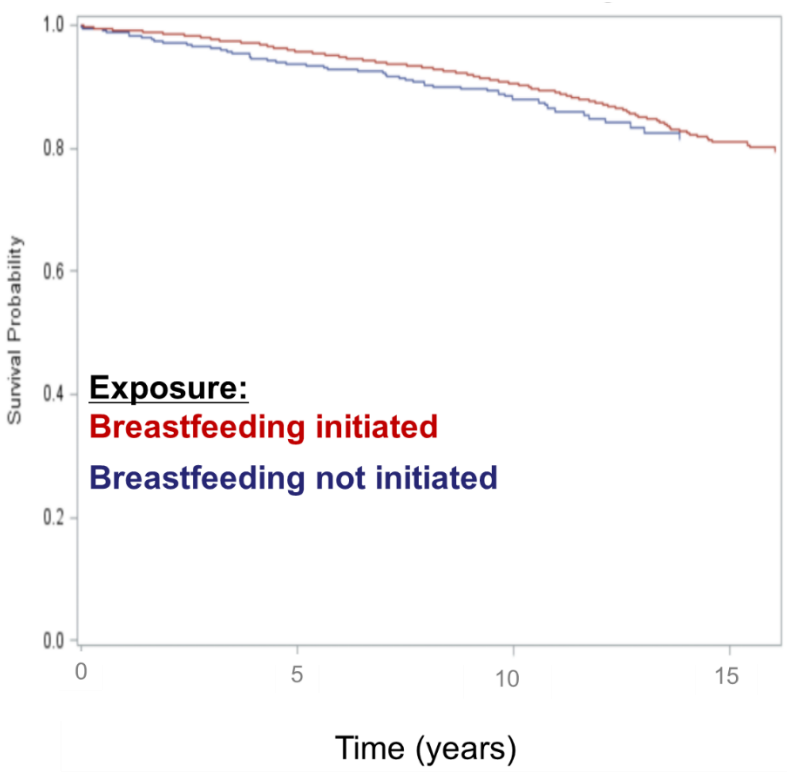
Crude Cox proportional hazards regression models revealed that breastfeeding initiation in offspring exposed to diabetes *in utero* reduced the incidence of the composite cardiovascular outcome compared to offspring not breastfed (HR 0.74, 95%CI 0.56-0.96). This protection was not observed among offspring not exposed to diabetes *in utero* (HR 0.95, 95%CI 0.85-1.07; Table 5.2). The protective effect of breastfeeding initiation among offspring exposed to diabetes was no longer significant after adjusting for confounding (aHR 0.91, 95%CI 0.69-1.20; Figure 5.2).

Table 5.3 Crude and fully adjusted hazard ratios (95% confidence interval) of the covariates for the composite cardiovascular outcome in offspring according to maternal diabetes exposure.

Predictor	Exposed to maternal diabetes		Not exposed to maternal diabetes	
	Crude HR (95%CI)	Adjusted HR (95%CI)	Crude HR (95%CI)	Adjusted HR (95%CI)
Male sex	0.71 (0.60-0.85)	0.67 (0.57-0.80)	0.86 (0.81-0.90)	0.82 (0.78-0.87)
SGA	1.08 (0.72-1.62)	1.15 (0.77-1.73)	1.11 (1.01-1.22)	1.09 (0.99-1.20)
LGA	1.63 (1.35-1.96)	1.44 (1.19-1.74)	1.11 (1.00-1.22)	1.11 (1.00-1.22)
Urban residence	0.49 (0.41-0.58)	0.75 (0.61-0.91)	0.91 (0.86-0.96)	1.11 (1.05-1.18)
Birth year	1.04 (1.01-1.08)	1.05 (1.01-1.08)	1.01 (1.00-1.02)	1.00 (0.99-1.01)
SEFI-2	1.46 (1.36-1.57)	1.24 (1.14-1.35)	1.23 (1.20-1.26)	1.12 (1.09-1.15)
Maternal age at birth	0.97 (0.95-0.98)	0.99 (0.97-1.00)	0.97 (0.96-0.97)	0.98 (0.98-0.99)

LGA: large for gestational age; SGA: small for gestational age; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status).

Figure 5.2 Adjusted survival curves for the composite outcome according to breastfeeding exposure, in offspring exposed to diabetes *in utero*.

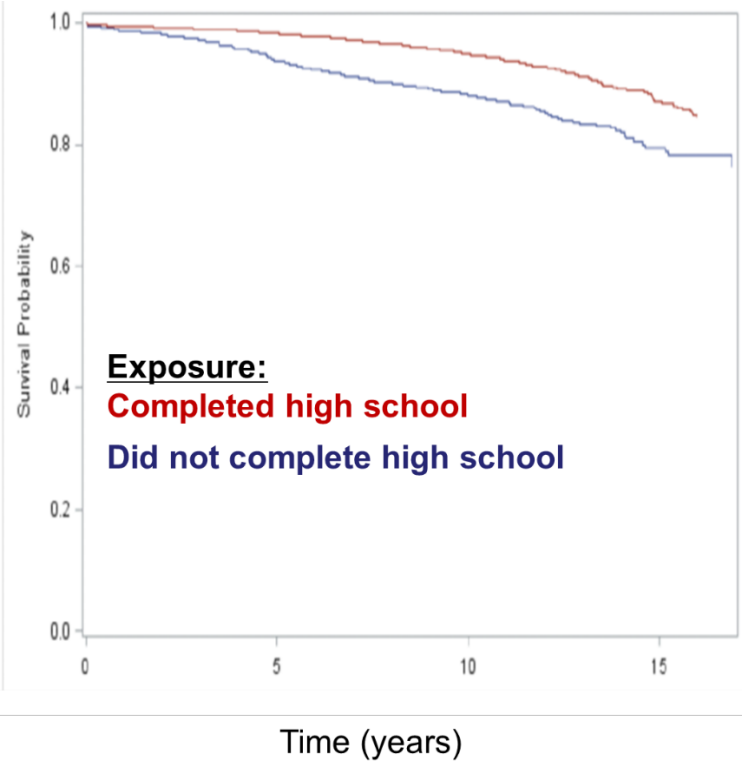


Red line is breastfed offspring; blue line is not breastfed (non-significant). Horizontal axis is time to event or censoring, starting from 10 years old (start of "at-risk" period) until up to 15 March 2015, in years.

High school completion and CVD Risk

In crude analyses, completing high school halved the risk of the composite CVD outcome compared to those who had not completed high school for offspring who were either exposed to diabetes *in utero* (HR 0.47, 95%CI 0.38-0.59) or not exposed (HR 0.54, 95%CI 0.51-0.58; Table 5.2). After adjustment for confounding, the effect size was reduced but remained significant (exposed to diabetes *in utero*: aHR 0.65, 95%CI 0.51-0.82; Figure 5.2). In the fully adjusted model, significant protective factors against the composite CVD outcome included male sex (aHR 0.67, 95%CI 0.57-0.80) and urban residence (aHR 0.75, 95%CI 0.61-0.91; Table 5.3). Factors that increased the risk for the outcome were lower socioeconomic status at birth (aHR 1.24, 95%CI 1.14-1.35), large birth weight for gestational age (aHR 1.44, 95%CI 1.19-1.74), and later birth year (aHR 1.05, 95%CI 1.01-1.08).

Figure 5.3 Adjusted survival curves for the composite outcome according to high school completion, in offspring exposed to diabetes *in utero*.



Blue line is offspring who did not complete high school; red line is those who completed high school. Horizontal axis is time to event or censoring, starting from 10 years old (start of "at-risk" period) until up to 15 March 2015, in years.

Do social determinants of health exert different effects according to exposure to diabetes in utero*?*

To test our secondary hypotheses around the interactions of breastfeeding, high school completion, and sex, we tested interaction terms in our model for offspring exposed to diabetes *in utero*. There was no significant interaction between breastfeeding and high school completion (Table 5.4). The interaction between sex and high school completion was significant only for males for whom high school completion information was missing (aHR 0.48, 95%CI 0.36-0.64 *vs* females with missing high school completion) while the estimates for the covariates did not

change meaningfully (Table 5.4). In analyses stratified by sex, significant protective predictors of the composite CVD outcome for females were high school completion (aHR 0.70, 95%CI 0.50-0.99), urban residence at birth (aHR 0.71, 95%CI 0.54-0.93) and higher maternal age at birth (aHR 0.98, 95%CI 0.96-0.99) whereas risk factors were low socioeconomic status at birth (aHR 1.34, 95%CI 1.19-1.51) and large birth weight for gestational age (aHR 1.65, 9%CI 1.29-2.12; Table 5.5). In males, the only significant protective factor was high school completion (aHR 0.60, 95%CI 0.42-0.84), whereas the only significant risk factor was later birth year (aHR 1.07, 95%CI 1.02-1.12). The complete case analysis did not change our conclusions about breastfeeding initiation not being significant and high school completion being significantly protective, but the loss in statistical power (n = 4626 offspring exposed to diabetes *in utero*) resulted in slightly different estimates (breastfeeding initiation aHR 0.78, 95%CI 0.55-1.12; high school completion aHR 0.70, 95%CI 0.54-0.90, Table 5.6). In the model replacing birth weight for gestational age by birth weight (continuous) and gestational age (preterm *vs* term), conclusions about breastfeeding and high school did not change, birth weight was not significant (aHR 1.00, 1.00-1.00) and preterm birth increased the risk for the outcome (aHR 2.18, 95%CI 1.69-2.82). Finally, in the model replacing birth year (continuous) by birth cohort (5-years categories), the estimates for breastfeeding and high school completion did not change significantly; CVD risk was significantly different only for offspring born in 1995-1999 *vs* those born in 1985-1989 (aHR 1.65, 95%CI 1.18-2.33). However, the goodness of fit was not superior in these two last models, so the original variables were kept in the main model.

Table 5.4 Fully adjusted hazard ratios (95% confidence interval) for the interaction terms between breastfeeding initiation and high school completion, and sex and high school completion

	Exposed to maternal diabetes Adjusted HR (95%CI)
Breastfeeding initiated vs not, did not complete high school	0.68 (0.44-1.11)
Breastfeeding initiated vs not, completed high school	0.70 (0.42-1.24)
Male vs female, did not complete high school	0.82 (0.58-1.15)
Male vs female, completed high school	0.83 (0.62-1.10)

Adjusted for missing high school completion values.

Table 5.5 Mutually adjusted hazard ratios (95% confidence interval) for the composite cardiovascular outcome in offspring, stratified by sex.

Predictor	Females Adjusted HR (95%CI)	Males Adjusted HR (95%CI)
Breastfeeding initiated	1.10 (0.74-1.66)	0.74 (0.50-1.07)
High school completion	0.70 (0.50-0.99)	0.60 (0.42-0.84)
SGA	0.85 (0.43-1.66)	1.42 (0.84-2.38)
LGA	1.65 (1.29-2.12)	1.22 (0.91-1.62)
Urban residence at birth	0.71 (0.54-0.93)	0.79 (0.59-1.06)
Birth year	1.03 (0.98-1.08)	1.07 (1.02-1.12)
SEFI-2 at birth	1.34 (1.19-1.51)	1.14 (1.00-1.29)
Maternal age at birth	0.98 (0.96-0.99)	1.00 (0.98-1.02)

LGA: large for gestational age; SGA: small for gestational age; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status).

Table 5.6 Complete case analysis. Crude and fully adjusted hazard ratios (95% confidence interval) for the composite cardiovascular outcome in offspring according to maternal diabetes exposure.

	Exposed to maternal diabetes		Not exposed to maternal diabetes	
	Breastfed	**Not breastfed**	**Breastfed**	**Not breastfed**
Cases/person-years	291/50 085	35/4229	4141/1 335 195	245/79 887
Cases per 1000 person-year	5.8	8.3	3.1	3.1
Crude HR	0.68 (0.48-0.96)	Reference	0.97 (0.85-1.10)	Reference
Predictor	**Crude HR (95%CI)**	**Adjusted HR (95%CI)**	**Crude HR (95%CI)**	**Adjusted HR (95%CI)**
Breastfeeding	0.68 (0.48-0.96)	0.78 (0.55-1.12)	0.97 (0.85-1.10)	0.99 (0.87-1.13)
High school completion	0.47 (0.68-0.59)	0.70 (0.54-0.90)	0.54 (0.51-0.58)	0.61 (0.56-0.65)
Interaction				
Breastfeeding initiated, did not complete high school	0.70 (0.42-1.24)	0.78 (0.47-1.34)	1.14 (0.89-1.49)	-
Breastfeeding initiated, completed high school	0.68 (0.44-1.12)	0.78 (0.50-1.30)	0.94 (0.81-1.09)	-
Male sex	0.84 (0.67-1.04)	0.82 (0.66-1.03)	0.85 (0.80-0.90)	0.82 (0.77-0.87)
SGA	1.09 (0.66-1.78)	1.13 (0.69-1.86)	1.11 (0.99-1.22)	1.08 (0.98-1.20)
LGA	1.55 (1.22-1.96)	1.36 (1.07-1.74)	1.10 (0.99-1.24)	1.13 (1.01-1.27)
Urban residence	0.54 (0.44-0.67)	0.73 (0.58-0.92)	1.00 (0.94-1.06)	1.11 (1.04-1.18)
Birth year	1.08 (1.03-1.13)	1.07 (1.02-1.11)	1.00 (0.99-1.02)	1.00 (0.99-1.01)
SEFI-2	1.46 (1.33-1.60)	1.31 (1.18-1.45)	1.20 (1.17-1.23)	1.10 (1.07-1.14)
Maternal age at birth	0.97 (0.96-0.99)	1.00 (0.98-1.02)	0.97 (0.97-0.98)	0.99 (0.98-0.99)

LGA: large for gestational age; SGA: small for gestational age; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status); urban: Winnipeg or Brandon.

Discussion

This registry-based birth cohort study with 28 years follow-up reported that completion of high school significantly reduced cardiovascular disease risk among offspring exposed to diabetes *in utero*. The protective effect of high school completion was similar in offspring not

exposed to diabetes. In contrast to previous studies of offspring risk for T2D, breastfeeding initiation did not confer protection against CVD outcomes in offspring, irrespective of intrauterine diabetes exposure status. The combination of breastfeeding initiation and completing high school did not confer additional protection against CVD outcomes, suggesting they might exert protection through similar pathways.

The results presented here support recent findings that reported small effects of breastfeeding initiation on markers of cardiovascular health in adolescence and young adulthood in the general population (no significant associations with blood lipids, blood glucose, or blood pressure, n = 5258, 10-18 years old[221]; risk of T2D aHR 0.83, 95%CI 0.69-0.99; n = 250 392, 7-24 years old[99]; dyslipidemia: mean total cholesterol difference = −0.18; 95% CI: −0.30 to −0.06 mmol/L, 6 studies, n = 1631, 17-64 years old[93]). One of the few studies that specifically focused on the effects of breastfeeding after intrauterine exposure to GDM showed a non-significant absolute reduction from 43.6% to 30.1% in the incidence of T2D in Pima adolescents 15-19 years old (n = 21[222]). Other cardiometabolic outcomes were not assessed in that cohort study.

Most studies that found a protective effect of breastfeeding on cardiovascular risk did not consider intrauterine exposure to diabetes as a risk factor. For example, the risk of death due to ischemic heart disease in older men was increased 1.2-fold among those bottle-fed compared to men breastfed for a maximum of 1 year (n = 5471, not independent of socioeconomic status[102]). Similarly, among 13-year-old adolescents that were born preterm, those fed banked breastmilk had lower systolic (4.2 mmHg) and diastolic (3.2 mmHg) blood pressure compared to preterm formula-fed infants (adjusted for sex, mean daily enteral sodium intake in infancy and current body mass index, but not socioeconomic status[223,224]). Finally, a systematic review revealed that breastfed adults displayed lower total and low-density lipoprotein cholesterol compared to those

that were bottle-fed (6 studies, n = 2014, unadjusted analyses[93]). As breastfeeding practices are highly correlated with socioeconomic status[99,102,103], the results of these studies are questionable as they did not control for income or other socioeconomic measures. Thus, although predictive statistical models projected a 25% reduction in CVD incidence if breastfeeding was promoted more broadly[223], the estimates they used (population-wide reductions between 0.18 mmol/L[93] and 0.30 mmol/L[225]) were not adjusted for socioeconomic status and potentially conflated the real lifetime cardiovascular protection that breastfeeding can confer to offspring. This may be why our results, carefully adjusted for socioeconomic variables, do not support an independent effect of breastfeeding initiation on cardiovascular risk up to 28 years after birth, irrespective of intrauterine exposure to diabetes.

Higher education attainment is associated with lower prevalence of CVD in adults[89,115,226], with effect sizes ranging between an unadjusted 6.7-fold difference in prevalence between non-high school graduates *vs* graduates[227] to an odds ratio of 2.64 (95%CI 2.16-3.22) after adjustments for age, sex, and race for elementary school graduates *vs* college graduates.[228] Using a retrospective cohort, Bell *et al*. did not find a significant association between high school completion and risk for hypertension, diabetes and hypercholesterolemia in 20-year olds participating in the National Health and Nutritional Examination Surveys (NHANES) between 2007 and 2014.[104] However, college completion was significantly protective towards hypertension with an effect size similar to ours after adjustment for current income (as well as other potential confounders including sex and age; odds ratio 0.72, 95%CI 0.59-0.89[104]). Our results extend the existing literature by prospectively evaluating the protective effect of high school completion in a population-based sample of offspring exposed to diabetes *in utero*. These

data provide an important public health target for attenuating future CVD risk among these offspring.

Contrary to our hypothesis, the combination of breastfeeding and high school completion did not lend additional protection towards CVD outcomes in offspring exposed to diabetes, potentially because of the small effect of breastfeeding compared to bigger predictors such as sex, urban residence, and socioeconomic status. The interaction term between sex and high school completion showed the sex difference in CVD risk was driven by those with missing high school completion values. As those with missing values were more likely to be male, breastfed, born at term in a rural area, have an adequate birth weight for gestational age, or not reach any of the endpoints in the composite outcome, it follows that this indicator would appear protective in the model. We can therefore conclude that there are no meaningful sex differences in our models. The lack of sex difference reinforces high school completion and educational attainment as a useful target to reduce or delay CVD incidence in the whole population.

Socioeconomic deprivation was an important predictor of CVD risk after intrauterine exposure to diabetes in all models, leading to a 24% increased risk of our composite outcome for each unit increase in SEFI-2 after adjustments. This supports previous observations that socioeconomic status in childhood is a reliable predictor of future disease risk, even after correction for current socioeconomic level.[98,229] Therefore, socioeconomic disparities remain an important risk factor to consider and address to increase health at the population level. Results from studies evaluating the impacts of minimum guaranteed income will clarify the benefits communities can expect from such measures aimed at reducing socioeconomic disparities.[230]

Policy recommendations

Our study highlights the importance of education, specifically high school completion, in reducing CVD risk incidence in offspring exposed to diabetes *in utero* (as well as in those not exposed to diabetes). As high school is attended after exposure to diabetes has happened, this suggests that governments can help improve the outcomes of the thousands of children and young adults who already have increased CVD risk while continuing to face the challenge of reducing rates of intrauterine exposure to diabetes. Socioeconomic status and urban residence, two additional factors that can be influenced by policies, remained important predictors of CVD after adjustment for sex and high school completion, highlighting the need for concerted actions between governmental departments (around Housing, Finances, Health, and Education for example) to effectively reduce the burden that CVD poses on our developed societies. Our study indicates benefits on CVD incidence appear as early as within the 10 years following high school completion, making interventions to increase high school completion politically viable.

Limitations

This study is not without limitations. First, our specific research question and lack of breastfeeding initiation data availability led to a restricted diabetes-exposed cohort which may not be representative of all Manitoban offspring exposed to diabetes *in utero*. The existence of a selection bias might lead to exaggerated effect sizes when compared to an unselected population. However, the similar effect sizes observed in the larger cohort of offspring not exposed to diabetes suggest that such bias would be small. Another limitation specific to administrative data is misclassification bias, which could impact the exposures (breastfeeding and high school

completion) and the outcome (CVD risk). However our breastfeeding and high school completion rates are comparable to, and slightly lower than, rates reported in the Canadian Community Health Survey and in the Canadian Census (breastfeeding: 91% in 2012[231]; high school completion: between 65.3% in 1996 and 80.2% in 2006[232]), increasing our confidence in these variables. While exclusive breastfeeding in hospital predicts longer breastfeeding duration[219,233], breastfeeding initiation is a limited marker of breastfeeding exposure. Future studies should also incorporate measures of duration and exclusivity. For the composite outcome, we adapted a validated algorithm as detailed in the Methods section to increase its specificity. Residual misclassification would bias our estimates towards nullity. Additionally, we were limited in our ability to capture other social determinants of health due to either lack of reporting in the administrative databases (for example ethnicity, which is not included in any administrative databases in the province), or to recent reporting, meaning that most cohort offspring would have missing values for these variables (such as housing data)[234]. While First Nations status can be linked to the Repository, ongoing discussions between the data holders (the Assembly of Manitoba Chiefs) and the Repository administrators (the Manitoba Center for Health Policy) are delaying the approval process, which prevented us from linking First Nations status to the present data in the context of this study. Likewise, conditions that were not diagnosed were not captured as part of our outcomes. It is therefore likely that offspring did develop our outcomes but have never been screened or diagnosed for them yet. As a large multi-country study indicated that around 50% of adults were unaware of having hypertension before being screened by the study team[207], the actual prevalence of our composite outcome might be higher than what we reported. As it is not possible to know if the underreporting of outcome would be different between groups, it is not clear whether this would bias our estimates towards

nullity or if it would artificially inflate our estimates. Finally, there could be residual confounding in our models due to unmeasured variables. We were not able to adjust our models for lifestyle (smoking, diet, physical activity) or medical (blood pressure, circulating glucose or lipids) variables that are known to impact CVD risk as they are not yet accessible in the Repository. Existing residual confounding would artificially inflate our current estimates.

Conclusions

In this registry-based cohort study, a statistically significant reduction in CVD incidence up to 28 years from birth was seen in offspring who completed high school, but not for those who were breastfed at birth, irrespective of intrauterine diabetes exposure. This was independent from modifiable and non-modifiable factors at birth. There was no significant interaction between high school completion and breastfeeding or high school completion and sex. Strategies to enhance high school completion rates might help reduce and delay CVD incidence in offspring exposed to diabetes *in utero*.

Acknowledgements

The authors acknowledge the Manitoba Centre for Health Policy for the use of data contained in the Manitoba Population Research Data Repository under project #Laetitia_thesis 2016040 (HIPC #2016/2017-06). 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 Ms. Yao Nie, Mrs. Heather Prior and Mrs. Kristine Kroeker for their logistical support in cutting the required data and their advice for analysing it.

Supplementary tables

Table 5.7 Diseases or risk factors classification and corresponding International Classification of Disease (ICD) codes, in alphabetical order.

Indication	ICD title	ICD 10 code	ICD 9 code
Diagnoses used in defining the intrauterine diabetes exposure			
Gestational diabetes	Diabetes mellitus in pregnancy, childbirth and the puerperium	O24	250
	Malnutrition-related diabetes mellitus	E12	250
Other diabetes	Other specified diabetes mellitus	E13	250
	Unspecified diabetes mellitus	E14	250
Type 1 diabetes	Insulin-dependent diabetes mellitus	E10	250
Type 2 Diabetes	Insulin-independent diabetes mellitus	E11	250
Diagnoses leading to exclusion from the cohort			
Cardiomyopathy	Endocardial fibroelastosis	I42.4	425.3
	Endomyocardial disease	I42.3	425.0
	Congenital cardiomyopathy	I42.8	Not applicable
	Familial cardiomyopathy	I42.9	425.9
Congenital malformation of the circulatory system	Congenital malformations of the circulatory system	Q20 to Q28	745, 746, 747, 759.9
Cystic fibrosis	Cystic fibrosis	E84	277.0
Ineligible diagnoses (not considered as an outcome, but did not warrant exclusion of the participant)			
Other cardiovascular problems (eg cause is viral, genetic, alcoholic, etc.)	Other cardiovascular problems	E78.7, E78.8	424.9, 425.2, 425.5, 425.7, 425.8, 425.9

Arrhythmia*	Cardiac dysrhythmias	I49	427 excluding 427.5 (see Cardiac arrest)
	Abnormalities of heartbeat	R00 to 03	785.3
Diagnoses indicative of a cardiovascular disease risk factor			
Type 2 Diabetes	Type 2 diabetes mellitus	E11	250
Other diabetes	Malnutrition-related diabetes mellitus	E12	250
	Other specified diabetes mellitus	E13	250
	Unspecified diabetes mellitus	E14	250
Hyperlipidemia	Dyslipidemia	E78	272.3, 272.5, 272.6
	Pure hypercholesterolaemia	E78.0	272.0
Hyperlipidemia	Pure hyperglyceridaemia	E78.1	272.1
	Mixed hyperlipidemia	E78.2	272.2
	Hyperlipidaemia, unspecified	E78.5	272.4
Atherosclerosis	Atherosclerosis	I70	440
	Essential (primary) hypertension	I10	401, 459.3
Hypertension	Hypertensive heart disease	I11	402
	Hypertensive renal disease	I12	403
	Hypertensive heart and renal disease	I13	404
Cardiomyopathy	Cardiomyopathy	I42.0, 142.1, 142.2, 142.5	425.1, 425.4
Cardiac risk factors	Abnormal findings on diagnostic imaging of the heart and coronary circulation	R93.1	793.99
	Abnormal results of cardiovascular function studies	R94.3	794.3
Prediabetes	Elevated blood glucose level	R73	790.2
Diagnoses indicative of a cardiovascular disease endpoint			

Ischemic heart disease	Angina pectoris	I20	413
	Acute myocardial infarction	I21	410, 434.91
	Subsequent myocardial infarction	I22	412
	Other acute ischaemic heart diseases	I24	411
	Chronic ischaemic heart disease	I25	414
Mitral valve problems	Nonrheumatic mitral valve disorders	I34	424.0, 394.0, 394.2, 394.9
Aortic valve problems	Nonrheumatic aortic valve disorders	I35	424.1, 424.2, 424.3, 396.0
Cardiac arrest	Cardiac arrest	I46	427.5
Heart failure	Heart failure	I50	428
Cerebral infarction	Cerebral infarction	I63	433, 434, 435
	Stroke	I64	437.0, 437.1, 437.3
Unspecified cardiovascular disease	Unspecified cardiovascular disease	I51.9	429.2, 429.9

*Arrhythmias were ineligible because their definition in administrative databases has not yet been validated and a local pediatric cardiologist informed us that most dysrhythmia diagnoses or referrals would not be considered cardiovascular disease or risk.

Table 5.8 Characteristics of the cohort according to breastfeeding exposure, stratified by sex, for offspring exposed to diabetes *in utero*.

	FEMALES		MALES	
	Breastfed N=2861	**Not breastfed N=274**	**Breastfed N=3152**	**Not breastfed N=364**
Maternal age at birth (years)	28.6 ± 5.7	29.0 ± 6.5	28.8 ± 5.8	29.0 ± 3.3
Birthweight (g)	3529 ± 581	3519 ± 765	3630 ± 610	3631 ± 809
LGA	643 (22.5)	80 (29.2)	778 (24.7)	115 (31.6)
SGA	114 (4.0)	17 (6.2)	172 (5.5)	25 (6.9)
Preterm birth	252 (8.8)	47 (17.2)	313 (9.9)	62 (17.0)
High school completed*	1557 (76.9)	130 (73.5)	1560 (71.3)	149 (70.0)
Urban resident	1738 (60.8)	132 (48.2)	1851 (58.7)	170 (46.7)
Birth year				
1985-1989	823 (28.8)	75 (27.4)	909 (28.8)	84 (23.1)
1990-1994	1398 (48.9)	95 (34.7)	1503 (47.7)	147 (40.4)

1995-1999	640 (22.4)	104 (38.0)	740 (23.5)	133 (36.5)
SEFI-2	0.41 ± 1.21	0.66 ± 1.26	0.45 ± 1.22	0.57 ± 1.27
Age at last follow up (years)	22.3 ± 3.0	21.5 ± 6.5	22.2 ± 3.1	21.5 ± 3.3
Age at first event (years)	17.8 ± 4.3	15.4 ± 3.8	16.7 ± 4.5	15.9 ± 4.6
Composite CVD outcome	260 (9.1)	26 (9.5)	198 (6.3)	33 (9.1)
Total incident CVD risk factors	253 (8.8)	26 (9.5)	184 (5.8)	31 (8.5)

Values are mean ± standard deviation or number (%). LGA: large for gestational age; SGA: small for gestational age; preterm birth: birth <37 gestational weeks; urban: Winnipeg or Brandon; SEFI-2: a measure of socioeconomic deprivation (higher index = lower socioeconomic status); CVD: cardiovascular disease.

*934 missing values for females, 1116 missing values for males.

6. Chapter 6. Intensive Gestational Glycemic Management and Childhood Obesity: A Systematic Review and Meta-Analysis

Authors: Laetitia Guillemette, Anita Durksen, Rasheda Rabbani, Ryan Zarychanski, Ahmed M. Abou-Setta, Todd A. Duhamel, Jonathan M. McGavock, Brandy Wicklow.

Manuscript status: Published in the *International Journal of Obesity (London)*, July 2017, Vol 41(7), pp 999-1004.

6.1. Contributions of Authors

I had the idea of this research question while taking the Systematic Review and Meta-Analysis class taught by Drs Zarychanski and Abou-Setta (co-authors) in the winter semester of 2016. I led the development of the protocol, search strategy (with advice from two librarians, Mrs. Angea Osterreicher and Becky Skidmore), and data extraction form. I conducted the search, citation review, and data extraction in duplicate with Mrs. Durksen. I did the quality assessment and data analysis; I wrote the draft manuscript and created all tables and figures with input from the co-authors at the stage of the protocol and of the manuscript writing. Hence, I am the sole first author on this paper. Mrs. Durksen was second reviewer, Dr. Rabbani provided statistical counsel, Drs Zarychanski and Abou-Setta led the class and provided input at all stages of the review, and Drs Duhamel, McGavock and Wicklow provided content expertise for the protocol and the manuscript.

6.2. Preface to the Manuscript

As detailed in section 1.1, diabetes (especially GDM) is one of the most common complications of pregnancy and can lead to many pregnancy and neonatal complications such as c-section delivery, neonatal hypoglycemia, shoulder dystocia, and macrosomia.[235] Studies indicating that screening for and treating GDM decreased the risk of complications in mothers and children were used to justify the implementation of universal GDM screening with the aim of preventing gestational and neonatal complications.[123,236,237]

In a series of subsequent papers, clinicians and researchers observed a linear relationship between maternal glycemia and risk of offspring metabolic complications such as macrosomia.[123,238,239] As this association was evident with serum glucose levels below the threshold for a GDM diagnosis, they hypothesized that initiating treatment of hyperglycemia below the GDM diagnostic threshold could further reduce the risk of offspring obesity.[123,237] It has thus been proposed that reducing current diagnostic thresholds for GDM – to effectively diagnose and treat more pregnant women – could be an effective way to curb the rising tide of childhood obesity.[240,241] However, others argued that this would put further strain on thinly spread healthcare systems and that imposing such an intensive "treatment" on otherwise healthy pregnant women would bring more harms than benefits.[242,243] Also, no one had evaluated the long-term impact of treating mild gestational hyperglycemia on childhood obesity in a systematic manner. We undertook a systematic review and meta-analysis to provide high-quality evidence to this debate.

This manuscript addresses our working hypothesis 2: Prenatal and/or postnatal social determinants of health can modify the relationship between intrauterine exposure to diabetes and offspring CVD risk. Three specific hypotheses will be tested:

2c. Intensive management of gestational hyperglycemia will reduce the risk for offspring obesity in childhood.

2d. Intensive management of gestational hyperglycemia will improve the offspring's metabolic profile as measured by waist circumference, adiposity, and weight-related indices.

2e. Intensive management of gestational hyperglycemia will be safe, and benefits will outweigh harms.

6.3. Manuscript

Abstract

Background and objectives: Hyperglycemia in pregnancy is associated with increased risk of offspring childhood obesity. Treatment reduces macrosomia; however, it is unclear if this effect translates into a reduced risk of childhood obesity. We performed a systematic review and meta-analysis of randomized controlled trials to evaluate the efficacy and safety of intensive glycemic management in pregnancy in preventing childhood obesity.

Methods: We searched MEDLINE, EMBASE, CENTRAL, and ClinicalTrials.gov up to February 2016 and conference abstracts from 2010 to 2015. Two reviewers independently identified randomized controlled trials evaluating intensive glycemic management interventions for hyperglycemia in pregnancy and included four of the 383 citations initially identified. Two reviewers independently extracted trial-level data with piloted forms and evaluated the internal validity of included studies using the Cochrane Collaboration's Risk of Bias tool. Data were pooled using random effects models. Statistical heterogeneity was quantified using the I^2 test. The primary outcome was age- and sex-adjusted offspring obesity measured in childhood. Secondary outcomes included offspring waist circumference and weight in childhood and maternal hypoglycemia during the trial (safety outcome). All outcomes were specified before the start of the review.

Results: The four eligible trials (n = 767 children) similarly used lifestyle and insulin to manage gestational hyperglycemia. We found no association between intensive gestational glucose management and childhood obesity at 7-10 years of age (relative risk 0.89, 95% CI 0.65 to 1.22;

2 trials; n = 568 children). Waist circumference also did not differ between treatment and control arms (mean difference −2.68 cm; 95% CI −8.17 to 2.81 cm; 2 trials; n = 568 children).

Conclusions: Intensive gestational glycemic management is not associated with reduced childhood obesity in offspring, but randomized data is scarce. Long-term follow up of trials should be prioritized, and comprehensive measures of childhood metabolic risk could be considered as outcomes in future trials.

Prospero Registration Number: CRD42016038624

Introduction

Childhood obesity[244] is a global public health concern[245] and correlates with important comorbidities such as type 2 diabetes in adolescence and early adulthood.[246,247] A recent systematic review of cohort studies from 188 developed and developing countries found that the prevalence of obesity in children aged 2 to 17 years nearly doubled between 1980 and 2013.[248] In North America, 12 to 17% of children are living with obesity.[249,250] Accordingly, the prevention of childhood obesity has been a World Health Organization (WHO) priority since 2012.[245]

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy[212], increases the risk for macrosomia at birth and obesity, metabolic syndrome and type 2 diabetes in childhood.[17,71,251,252] Previous systematic reviews indicate that GDM is an independent risk factor for macrosomia[253], but glycemic management reduces the risk for macrosomia and large for gestational age newborns.[213] Two benchmark cohort studies found that maternal hyperglycemia in pregnancy linearly increases the risk for fetal macrosomia, without a clear glucose inflection point.[18,238,240,254] As such, it is widely accepted that women at risk of GDM should target normal glycemia during pregnancy[13,239,255,256] and that this could reduce the risk of childhood obesity[257,258]. However the degree to which these recommendations are based on evidence is uncertain.

The objectives of this systematic review were to identify, critically appraise, and meta-analyze data from prospective randomized trials comparing, in pregnant women, the impact of intensive maternal glycemic management *vs* standard of care on offspring obesity risk in childhood.

Materials and methods

Using an *a priori* published protocol (PROSPERO #CRD42016038624), we conducted our systematic review using methodological approaches outlined in the *Cochrane Handbook for Systematic Reviews*[259] and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline.[260,261] A technical panel of experts from multiple fields (endocrinology, developmental origins of diseases, research methodology) formulated the review question, reviewed the search strategies and review methods, and provided input throughout the review process.

Data Sources and Searches

We searched the following electronic databases from inception to February 2016: Medline (Ovid), EMBASE (Ovid), and CENTRAL (the Cochrane Library – Wiley). All searches were conducted between February and April 2016. The Cochrane Highly Sensitive Search Strategy was used as a model for searching[259], and we designed search strategies specific to each database. The search strategy for Medline is presented as an example online in the PROSPERO registration document. To identify ongoing or planned trials, we also searched the WHO's International Clinical Trials Registry Platform. In addition to electronic searching, we hand-searched abstracts and conference proceedings of the following societies (2010-2015): *American Diabetes Association*, *Canadian Diabetes Association*, *European Association for the Study of Diabetes*, *International Diabetes Federation*, *American Congress of Obstetricians and Gynecologists*, *Society of Obstetricians and Gynaecologists of Canada*, and the *Royal College of Obstetricians and Gynaecologists*. Lastly, we hand-searched the bibliography of relevant

narrative and systematic reviews for additional relevant citations. Reference Management was performed in EndNote™ (ver. 16, Thompson Reuters).

Study Eligibility

Inclusion criteria We included only randomized controlled trials of pregnant women diagnosed with mild GDM, glucose intolerance or hyperglycemia of pregnancy, according to local definitions and diagnosis criterion. This means that the criterion used to diagnose "mild" GDM in one center could have been used to diagnose "actual" GDM in a different center. Pharmacological and non-pharmacological interventions were accepted, regardless of timing or regimen.

Exclusion criteria We excluded all non-randomized study, trials lacking an untreated comparator group, and trials in which women were known to have diabetes before pregnancy and/or for which data was not presented for women with (new) gestational hyperglycemia as a subgroup. No other restrictions, including language or publication status, were considered.

Study selection We used a two-step process for study selection. First, two reviewers (LG and AD) independently screened the titles and abstracts (when available) of each search result to determine if a study met the general inclusion criteria. The same two reviewers independently examined the full-texts of relevant citations. Disagreements were resolved by discussion between the two reviewers or by third-party adjudication (BW), as needed. Study authors were contacted if data as published was incomplete for the needs of the review.

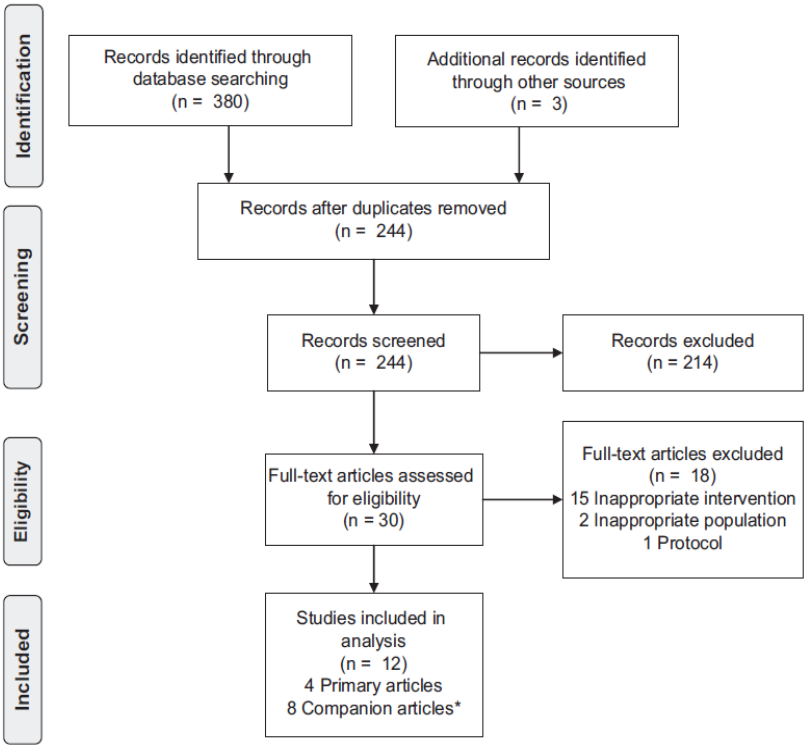
Study Design, Interventions of Interest and Outcome Measures Our primary research question was: "in women diagnosed with hyperglycemia in pregnancy, is intensive glycemic management of hyperglycemia to achieve or approach normoglycemia during pregnancy associated with reduced measures of offspring obesity, compared to standard of care?". We performed a meta-analysis evaluating trials that tested *intensive glycemic management* defined as initiating glycemic management at glycemic levels below locally approved GDM diagnostic criterion, compared to standard of care. **Our primary outcome measure** was the incidence of age- and sex-adjusted offspring obesity according to the local reference growth chart, measured between 1 and 17 years of age. Secondary outcomes were offspring waist circumference, BMI z-score, fat mass, and weight at longest follow up. To evaluate the safety and acceptability of this intervention for preventing childhood obesity, we also assessed maternal outcomes including hypoglycemia during the intervention period and health-related quality of life.

Data Extraction and Quality Assessment

Two reviewers (LG and AD) independently extracted data from included trial reports using a standardized and piloted data extraction form. Discrepancies between the two reviewers were resolved through discussion with a third reviewer (BW). Extracted data included *funding sources*, *demographics* of the enrolled mothers and children (gestational age at randomization, maternal age at randomization, pre-pregnancy BMI, maternal smoking status at randomization; child age at latest follow-up), *details of the glucose management intervention* (glucose level target of glycemic management, number of planned visits with research healthcare professionals, number of daily capillary glucose tests recommended, glucose management prescribed: lifestyle,

oral hypoglycemic medications, insulin), and predetermined relevant maternal and offspring *outcomes* as described above. When a trial reported results for more than one period, only results from the longest follow up were included. Data management was performed using Microsoft Excel 2007 (Microsoft Corp). Internal validity of included studies was evaluated using the Cochrane Collaboration's Risk of Bias tool[262] which assesses randomisation and allocation of participants; blinding of participants, personnel, and outcome assessors; incomplete or selective reporting; and other relevant sources of bias. If trial methodology was unclear from the published report, authors were contacted for clarification.

Figure 6.1 Flow diagram of literature search and study selection.



According to the 2009 Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) with modifications. *Companion articles represent reports involving the same study population for the same intervention trial, including conference abstracts reporting findings from previous or subsequent full-length publications.

Data Synthesis and Analysis

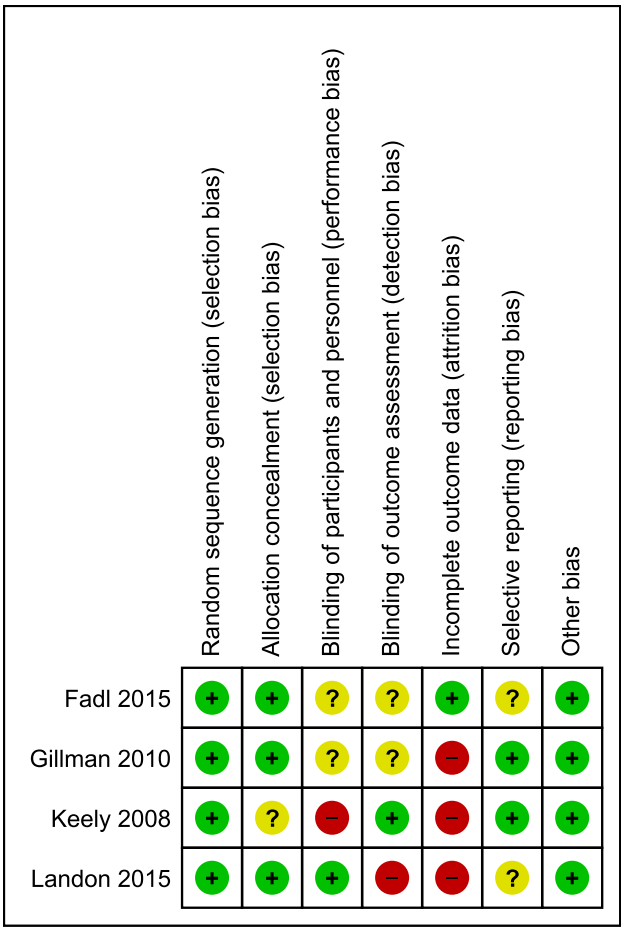
We analyzed data from the included studies using Review Manager (RevMan version 5.3.5, the Cochrane Collaboration). Pooled dichotomous data are presented as risk ratio (RR), and pooled continuous data are expressed as mean difference (MD), with 95% confidence intervals (CI). Statistical heterogeneity was explored and quantified using the I^2 test. Publication bias was not assessed due to the small number of trials included. After validation against the associated protocols and/or trial registration information, no evidence of selective reporting was found for any of the included trials.

Results

Of 383 citations identified from electronic and hand-searches, we included four unique randomized trials (plus eight companion publications), representing a total of 767 children between 4 and 10 years old (Table 6.1, Figure 6.1). All were randomized controlled trials published in peer-reviewed journals between 1997 and 2015 and conducted in developed countries: Australia[258], Canada[263], Sweden[264], and United States of America.[240] Two trials[258,264] enrolled women with at least one risk factor for GDM, while the other two[240,263] did not select their participants based on GDM risk factors. All trials excluded women with diabetes diagnosed before pregnancy or multiple pregnancies. Details of the glucose management intervention conducted in each trial are presented in Table 6.2. All trials were adjudicated to be at unclear or high risk of bias, mostly due to the unclear effectiveness of blinding and to high attrition rates between the trial initiation and the follow up of children experienced by all studies (Figure 6.2). The diagnostic criterion for inclusion was slightly different between trials, which

might influence the level of hyperglycemia experienced by the participants (Table 6.2). Mean maternal age was 30.2 ± 5.3 years, and between 28%[240] and 90%[258] of participants were of Caucasian descent. In all trials, women in the intervention arm attained glycemic targets. In the sole trial that measured both the treatment and control arms' glycemia at the end of pregnancy[263], the mean difference in glycemia between groups was minimal (0.21 ± 0.93 mmol/L for fasting glucose, and 0.51 ± 1.65 mmol/L one hour post-prandial at 36-38 gestational weeks).

Figure 6.2 Summary of risk of bias for individual studies following the Cochrane tool.



Low risk of bias is indicated by the plus sign, High risk of bias by the minus sign, and Unclear risk of bias, by the question mark.

Table 6.1 Characteristics of included trials and study populations, arranged according to duration of follow up.

Trial and Companion Articles	Country & Enrollment Period	Maternal Age at Randomization (yrs)	Number of Participants[a]	Offspring Age at Last Follow up (yrs)	Child Obesity Definition	Relevant Outcomes		
						Obesity	WC	FM
Keely 2008[83,85,86]	Canada, 1991-1994, 2001-2002	30.7±4.7	300 mothers, 68 children	7-11	>95th percentile of 2000 CDC growth chart, age- and sex-adjusted	●	●	-
Landon 2015[72,78,87-90]	USA, 2002-2007, 2012-2013	29.0±5.4	958 mothers, 500 children	5-10	≥95th percentile of 2000 CDC growth chart, age- and sex-adjusted	●	●	-
Gillman 2010[79,91]	Australia, 1993-2003; 1997-2007	30.5±5.5	1000 mothers, 199 children	4-5	≥85th percentile of 2000 IOTF growth chart, age- and sex-adjusted	●	-	-
Fadl 2008[84]	Sweden, 2008-2011	31.6±5.7	69 mothers, 0 children	-	-	-	-	-

CDC: Center for Disease Control and Prevention; FM: fat mass; GDM, gestational diabetes mellitus; IOTF: International Obesity Task Force; WC: waist circumference; Yrs: years. Data are mean ± standard deviation. [a]Number of pregnant women randomized, and number of children included in the follow-up study.

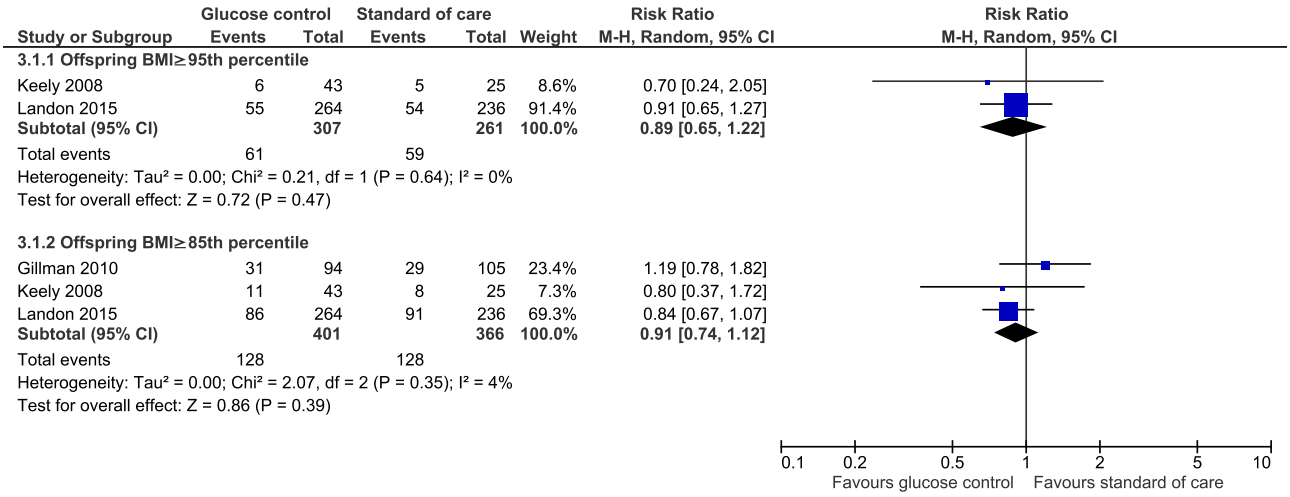
Table 6.2 Characteristics of glucose management interventions in included trials.

Trial	Glycemic threshold to initiate intervention (mmol/l)	Gestational age at randomization	Glucose control strategy	Failure Criteria[a] (mmol/l)
Keely 2008[85]	75g OGTT; 2h>7.5	24-32 weeks	Glucose testing 4 times daily, caloric restriction (35 kcal/kg of ideal body weight)	>2 values over target: FG<4.4, 1h PPG <7.8
Landon 2015[78]	100g OGTT; FG<5.3 and 2 of the following: 1h>10.0; 2h>8.6; 3h>7.8	24-31 weeks	Glucose testing 4 times daily, diet modifications	Majority of weekly values over target: FG <5.3, 2-hour PPG <6.7
Gillman 2010[79]	75g OGTT; FG<7.8, 7.8 < 2h < 11.0	28-30 weeks	Glucose testing 4 times daily, diet and exercise modifications	Over target after 2 weeks: FG<5.5, preprandial levels < 5.5, and 2h PPG <7.0
Fadl 2008[84]	75g OGTT; FG<7.0, 10.0<2hr<12.2	28-32 weeks	Glucose testing 4 times daily, diet modifications	>2 values over target: FPG<5.0; 2h PPG<6.5

FG: Fasting glycemia; GDM: gestational diabetes mellitus; OGTT: oral glucose tolerance test; PPG: post-prandial glycemia; 1h: glycemia 1 hour into the OGTT; 2h: glycemia 2 hours into the OGTT; 3h: glycemia 3 hours into the OGTT. [a]Glycemic criteria at which insulin is added to the glycemic management strategy.

For the primary outcome measure, two trials[240,263] involving 568 pregnant women (follow up 8.1 ± 1.1 years later) provided data for the meta-analysis of obesity in childhood (Figure 6.3). The incidence of obesity at longest follow up was not significantly different between groups (19.9% in the treatment arm vs. 22.6% in the standard care arm; RR 0.89, 95% CI 0.65 to 1.22; I^2 0%).

Figure 6.3 Forest plot of pooled risk ratios for childhood obesity after exposure to glycemic management in pregnancy or standard of care.



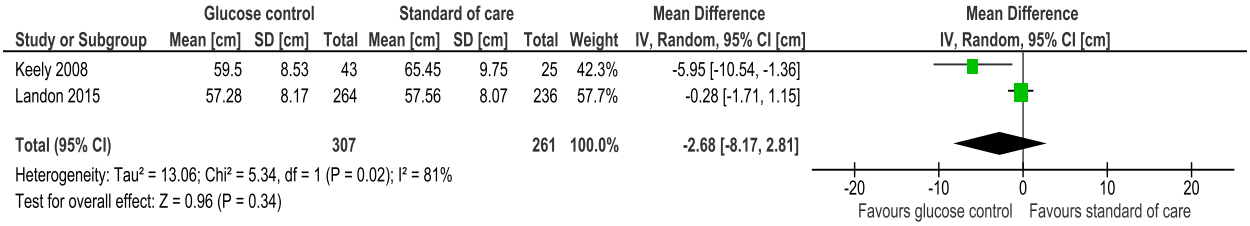
Height and weight were measured in 568 children at a mean age of 8.1 ± 1.1 years.

In a third trial[258] involving 199 pregnant women followed for 4.7 ± 0.3 years, intensive gestational glycemic management failed to reduce the proportion of children with a BMI ≥85th percentile of the 2000 International Obesity Task Force (IOTF) growth chart.[265,266] Because BMI ≥85th percentile aligns more closely with a classification of overweight than obesity[267], we considered it to be clinically different from the previous outcome (BMI ≥95th percentile of the 2000 CDC growth chart) and thus present it as a sub-group analysis. Among the 767 children (5.8 ± 0.6 years old) with available data, the incidence of BMI ≥85th percentile was 31.9% in the treatment arm *vs* 35.0% in the standard care arm (RR 0.91, 95% CI 0.74 to 1.12, I^2 4%, Figure 6.3).

For waist circumference, the only secondary outcome reported by more than one study, two trials[240,263] involving 568 pregnant women (follow up 8.1 ± 1.1 years later) provided data for meta-analysis. Intensive gestational management of hyperglycemia was not associated with

significant changes in mean waist circumference in children (58.4 ± 8.4 cm in treatment arm vs 61.5 ± 8.9 cm in standard care arm; MD −2.68, 95% CI −8.17 to 2.81 cm, I^2 81%, Figure 4). While trials appeared to be clinically similar, statistical heterogeneity was high, possibly because of the large confidence intervals associated with the measurements.

Figure 6.4 Forest plot of mean difference for childhood waist circumference after exposure to glycemic management in pregnancy or standard of care.



Weights are from random-effects analysis. Waist circumference was measured in centimetres (cm) in 568 children at a mean age of 8.1 ± 1.1 years.

Discussion

We found no conclusive evidence from the small number of published randomized trials that intensive management of gestational hyperglycemia reduces childhood obesity even if it is successful in reducing the risk for macrosomia in newborns. There was also no significant reduction in offspring waist circumference, and there was insufficient data to evaluate other indicators of childhood metabolic health or maternal safety (such as hypoglycemic events). Additionally, we observed that there are very few clinical trials of intensive management of gestational hyperglycemia investigating the risk of offspring obesity in childhood.

Current clinical practice guidelines advocate normalizing glycemia in high-risk pregnant women to reduce the risk of pregnancy-related complications, newborn macrosomia, and child

obesity.[11,16,268] While clinical trials have established the usefulness of this approach for the first two outcomes, high-quality evidence is scarce for the impact of normalizing maternal hyperglycemia on the long-term effects of childhood obesity.[269,270] The four trials included in this systematic review observed a reduced risk for macrosomia among pregnant women with hyperglycemia, concurring with recent systematic reviews that confirmed treatment of hyperglycemia reduces the relative risk of macrosomia by half (95% CI, 0.38–0.57)[212,213]; however, these benefits did not translate into a reduced risk for obesity later in childhood.

Investigators have previously called for more trials of glycemic management among high-risk women without GDM that include appropriate follow-up of offspring into childhood.[17,124,252] Our results support these calls and reinforce the need for long-term follow-up of offspring exposed therapeutic trials during pregnancy before undergoing costly reforms of clinical care and policies for currently low-risk women. We add that follow-up should be conducted systematically between 1 and 17 years after birth; efforts should be made to limit loss to follow-up and/or sample sizes should be at least 220 offspring per exposure group (to reach a significance level of 0.05 and power of 80% specifically for the outcome of obesity prevalence); and outcomes should include metabolically-relevant measures such as waist circumference and fat mass as well as maternal safety and quality of life outcomes. Indeed, although several trials of glucose management in women in mild GDM reported a reduced risk of macrosomia or large-for-gestational-age infants[212,213], few of those trials followed the offspring into childhood to determine if this lowered risk translated into a lower risk of obesity and other cardiometabolic outcomes. Moreover, of the four trials included in the current systematic review, all were at similar risk of bias due to unclear blinding to intervention group and to attrition between the trial and the follow-up study. Finally, the lack of maternal outcomes reported is troubling considering

that proposed lowering of current GDM diagnostic thresholds to capture low-risk women would undoubtedly have important impacts on them.

The strengths of this review include the extensive search strategy used, the multiple databases and trial registries searched, and the hand-search of citations in selected conference proceedings. The included trials were clinically homogenous in their populations, interventions, and potential biases. Finally, we used a protocol published *a priori* and complied with established methodological guidelines in the conduct and reporting of this review. Limitations include pooling data identified at high risk of bias due to attrition, the limited number of included studies, and the lack of representativeness towards non-developed countries with possibly different healthcare systems.

Currently, there is insufficient experimental evidence to support intensive glycemic management of gestational hyperglycemia below current GDM diagnostic threshold to prevent childhood obesity in the offspring. Longitudinal follow-up of children involved in therapeutic trials of mild GDM and GDM are required. To determine if intensive maternal glycemic management improves metabolic health into childhood, other markers of metabolic risk (eg fat mass and dyslipidemia) should also be included as outcomes in future clinical trials; to determine the acceptability of this intervention for childhood obesity, maternal outcomes (eg. hypoglycemia and quality of life) need to be evaluated and reported.

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7. Chapter 7. Discussion

Given the increasing rate of exposure to diabetes i*n utero*[2,271] and the decreasing age at which CVD occurs in ever larger numbers worldwide[78,272], the current dissertation was guided by two central research questions: is intrauterine exposure to diabetes a risk factor for early CVD risk? If so, are there social determinants of health that can attenuate this risk? Chapters 3 to 6 present the results from four studies that were designed to answer these two overarching questions. To answer the first question, chapter 3 investigated the effects of intrauterine diabetes exposure on pre-clinical markers of CVD risk (LV function and morphology) in a high risk pediatric cohort, and chapter 4 expanded this question to a broader population and determined if intrauterine diabetes exposure was associated with an increased incidence of established CVD risk factors (hypertension, dyslipidemia, etc.) and CVD endpoints. To answer the second question, chapter 5 determined if breastfeeding and high school completion were associated with lower CVD risk even after exposure to intrauterine diabetes at the population level, and chapter 6 determined through a systematic literature review if intensive glycemic management of hyperglycemia in pregnancy could reduce the risk for childhood obesity, an important risk factor for early CVD. Together, these studies propose complementary evidence to inform the DOHaD theory and the two overarching questions of this dissertation.

7.1. Overview of Findings

The evidence created and collated in this dissertation supports and extends the global DOHaD theory suggesting that the intrauterine environment can programme chronic disease risk. Indeed, we show that the risk for CVD was elevated in young people exposed to diabetes *in utero*. Additionally, we found that certain determinants of health are associated with lower CVD

risk following intrauterine diabetes exposure. These findings could have direct applications in both clinical and policy settings to change, on the one hand, how clinicians follow and screen patients for CVD and, on the other hand, how public health policies and government programs can be tailored to buffer long-term CVD risk with targeted prevention and treatment initiatives at the population level. Inspired by the theoretical framework underlying this dissertation (Figure 2.1), the next sections are going to address 1) how our findings inform the DOHaD theory; 2) how specific social determinants of health influence the relationship between intrauterine diabetes exposure and CVD risk; and 3) future directions.

7.2. Contributions to the DOHaD Theory

7.2.1. Intrauterine Diabetes Exposure as a Novel Risk Factor for CVD

Among youth living with T2D, we found that those exposed to diabetes *in utero* displayed higher 24 h blood pressure loads and a trend towards impaired LV filling and a reduced LV size, compared to peers not exposed to diabetes *in utero*. At the population level, we found that intrauterine exposure to GDM or pre-existing diabetes was associated with a dose-dependent increased incidence of CVD risk factors and endpoints. We also found that this risk presented at an earlier age than in those not exposed to diabetes *in utero*. The population-based study from chapter 4 clarified that GDM exposure nearly doubles – and exposure to pre-existing diabetes triples – the risk of receiving a diagnosis related to poor cardiovascular health within 35 years of life. In fact, the studies from chapters 3 and 4 complement each other to paint a clearer picture of the early CVD risk associated with intrauterine exposure to diabetes. An important finding consistent between these two studies is that offspring exposed to diabetes *in utero* are

significantly more likely to develop hypertension, both in adolescents and young adults living or not with T2D (Tables 3.1 and 4.1). Hypertension is a long-established dominant risk factor for CVD in adults[273], but not in pediatric populations[274,275] even if prevalence is higher than previously estimated among young people.[276,277] This absence in pediatric guidelines should mayhap be revisited, considering that blood pressure in childhood and adolescence tracks into adulthood (reviewed in [278]) and hypertension is a major risk factor specifically for early CVD.[79] In this dissertation, we found that more than half (56.8%) of the adolescents with T2D (Table 3.1) and 2.5% of 10 to 35-year-olds in Manitoba (Table 4.1) had been diagnosed with hypertension. These rates are higher than those reported in the 2007-2009 Canadian Health Measures Survey in which only 1% of Canadian children (6-19 years old) had hypertension.[279,280] We are the first to show that intrauterine exposure to diabetes substantially increases the risk for hypertension in adolescence and young adulthood, both in clinical and population-based cohorts (indirectly for the latter, as hypertension accounts for 61% of the composite outcome and intrauterine diabetes exposure increased risk for the composite outcome).

While novel, our results are founded on work published more than 30 years ago by Barker and Freinkel as mentioned in section 1.2.2. Our data contribute to joining their two hypotheses by conducting studies with reliable assessments of intrauterine diabetes exposure and gold-standard measures of early CVD risk with reasonably large sample sizes.[272] Additionally, while scientific research and public interest around the "Barker hypothesis" were focused on the impacts of fetal exposure to deprivation (reviewed in [49,281]), comparatively few studies are centred on fetal overnutrition like ours. It may be because fewer pregnancies were complicated by T2D or GDM in that period than today.[2,271] The recent parallel increase in the incidence of

intrauterine exposure to diabetes and early CVD risk has intensified the interest in determining if these conditions are connected. Recent findings in this area illustrate how this exposure is associated with higher rates of offspring T2D, overweight/obesity, high blood pressure (below hypertension levels), and dyslipidemia.[69,169,282] While animal models replicating the exposure to diabetes *in utero* support the hypothesis that this exposure increases the prevalence of CVD risk markers (reviewed in [68,283,284]), its impact on CVD endpoints in humans had yet to be clarified.

Data from chapters 3 and 4 fill this knowledge gap. Both studies revealed that intrauterine exposure to diabetes was associated with high rates of CVD risk factors and endpoints, specifically T2D and hypertension, and this association was apparent in adolescence (14 and 19 years old on average). On the one hand, there is an abundance of evidence that intrauterine diabetes exposure is associated with risk for T2D in human offspring (reviewed in [23]) and this was also found in our study. The specific mechanisms underlying this association are being elucidated in animal models (reviewed in [285]). On the other hand, some studies suggest blood pressure is higher in offspring exposed to intrauterine diabetes both in humans[282,286–289] and animals[129,198], but evidence is scarce[43] and does not link this exposure to established hypertension. The results in this dissertation add substantial support to this end by providing estimates with high precision (using 24 h ambulatory blood pressure monitoring) and population-level longitudinal data. The novelty of our findings is specifically tied to the association with hypertension. At the population level, this association remained significant after adjustment for other factors related to elevated blood pressure and hypertension: birth weight (reviewed in [54,290]), small[291] or large[292] birth weight for gestational age, preterm birth [291,293], and socioeconomic status.[294] When we restricted the composite outcome to CVD endpoints (thereby excluding CVD risk factors such as hypertension and T2D), the strength of the association

between intrauterine diabetes exposure and CVD incidence was reduced for both GDM and pre-existing diabetes exposures, but remained statistically significant (Table 4.2 and section 4.3.4). This might imply that intrauterine diabetes exposure is more directly related with early CVD risk factors such as T2D and hypertension or that this reduced association is due to the limited number of offspring who reached a CVD endpoint (n = 1769).

In conclusion, our results contribute to the limited evidence supporting an association between intrauterine exposure to GDM and pre-existing T2D and risk of CVD. They also highlight adolescence as a potentially important period during which CVD risk solidifies. This hypothesis is briefly discussed below.

7.2.1.1. *Adolescence as an important period for CVD risk*

As presented in section 1.2, the DOHaD theory posits that part of CVD risk might originate *in utero* and environmental cues might mediate this risk either during critical windows or throughout life. Using either viewpoint (critical windows or life course approach), adolescence may be considered as an important period in defining CVD risk. Indeed, it is typically during adolescence that children gain autonomy over their behaviours and begin or discontinue lifestyle habits that may be harmful (such as smoking, eating out, stopping extracurricular sports) or beneficial (such as cooking from fresh ingredients, using active modes of transportation, creating and relying on networks of peers).[295,296]

Applying the "life course approach" to the results of chapter 3, it is possible that we measured LV function too early and that the participants' various risk factors, including intrauterine diabetes exposure, had not had time to compound each other in a manner meaningful

enough to be detected by Doppler echocardiography. Thus, the lack of association between intrauterine diabetes exposure and LV dysfunction would be due to the young age of the participants, and repeated measures (after adolescence) might yield different conclusions. Our results from chapters 4 and 5 also support adolescence as an important period, considering that the average age at first CVD event was 19 and 16 years old in offspring exposed to GDM and pre-existing diabetes *in utero*. Additionally, lifestyle changes and upward socioeconomic mobility during or after adolescence have been associated with reductions in blood pressure in early adulthood (26-36 years old[297]), suggesting that changes undergone during adolescence can influence the lifetime cardiovascular risk trajectory. The critical windows theory would emphasize the use of screening and interventions during the pubertal period to optimize the reconditioning of the CVD risk associated with intrauterine diabetes exposure.

7.3. Social Determinants of Health as Modifiers of CVD Risk

7.3.1. Mediating Factors in the Association between Intrauterine Diabetes Exposure and CVD Risk

Considering the growing body of evidence supporting an association between intrauterine diabetes exposure and higher risk of early CVD, it is important to identify modifiable factors that can reduce this risk. Indeed, while preventing intrauterine exposure to diabetes altogether is an excellent goal for primordial prevention, CVD prevention and treatment strategies also need to be identified for the thousands of infants exposed to diabetes *in utero* every year.[171] As the health of individuals is inextricably related to the health of the population they evolve in[298], the studies in chapter 5 and 6 focused on interventions that could be applied to populations through policy changes rather than on generally accepted but individualistic CVD prevention strategies

(such as smoking cessation or modifications in diet and exercise patterns[299,300]). For example, if breastfeeding initiation had been found to significantly reduce CVD risk in offspring exposed to diabetes *in utero*, we could have recommended the promotion of breastfeeding initiation specifically to women with diabetes. Our findings indicate that the increased CVD risk following intrauterine exposure to diabetes is modifiable. Initiating breastfeeding at birth (table 5.2) and intensively treating mild gestational hyperglycemia (figure 6.3) were both associated with a non-significant ~10% lower CVD risk whereas high school completion was associated with a significant 35% lower risk in offspring exposed to diabetes *in utero*. Our test for a potential interaction between breastfeeding initiation and high school completion did not support the hypothesis that these two factors had additive protective effects against early CVD after exposure to diabetes *in utero*. This highlights that although the relationship between intrauterine diabetes exposure and CVD is modifiable, not all intervening factors exert similar effects. This might be due to the extent or nature of the intervening factor, or to the severity of the exposure, as these parameters were different between all three intervening factors tested. The next three sections will elaborate on each of these factors individually.

7.3.1.1. *Breastfeeding as a protective factor against CVD risk*

In contrast to our *a priori* hypothesis and some of the previous work in the area of breastfeeding, we did not observe an association between breastfeeding initiation and CVD risk in offspring. This lack of association was surprising as a previous study using the same variable and a similar population found a statistically significant 17% reduction in hazards for early-onset T2D in offspring (aHR 0.83, 95%CI 0.69-0.99).[99] A major difference between this study by

Martens and ours was the outcome, suggesting the protective effects of breastfeeding initiation might not extend similarly to different CVD risk factors. Alternatively, breastfeeding practices are reliably associated with socioeconomic status.[102,103] Contrary to Martens, I used the socioeconomic indicator SEFI-2 instead of income as it includes measures of social deprivation (neighbourhood-level unemployment, education attainment, single-parent households). It is possible that if Martens *et al*. had also corrected for SEFI-2 and not simply income, they would have observed a similar non-significant result as their estimate had relatively low precision (0.69-0.99). Statistical significance aside, Martens' study also obtained a larger effect size (17%) than ours (9%), indicating that breastfeeding might be more protective against glycemic dysfunctions than against cardiovascular dysfunctions. Animal studies cannot settle these conflicting results as they generally suggest that the suckling period increases metabolic risk in models of offspring exposed to diabetes or obesity *in utero* (reviewed in [67,68,301]). Future studies need to investigate and report the effects of breastfeeding on CVD risk separately for offspring exposed or not exposed to diabetes *in utero* while accounting for socioeconomic status. In addition, duration and exclusivity should also be investigated to gain a fuller picture of the effect of breastfeeding on CVD risk after intrauterine exposure to diabetes.

7.3.1.2. *High school completion as a protective factor against CVD risk*

In agreement with our hypothesis, we provide novel data demonstrating that high school completion was significantly associated with lower CVD risk in offspring exposed to diabetes *in utero* (Table 5.2). This is consistent with current evidence on the cardiometabolic benefits of formal education as detailed in section 5.4. More specifically, education might be protective

towards cardiometabolic diseases through direct and indirect effects. Direct effects include improvement of cognitive skills in understanding abstract concepts such as "healthy lifestyle", increased mental abilities to follow health prescriptions and adopt healthy behaviours, and the development of literacy and numeracy skills that can then be applied to health literacy.[302–304] Indirect effects include increased opportunities for employment and higher earnings[116], increased ability to navigate complex healthcare systems[305,306], and access to social resources such as supportive familial and peer networks[307], which themselves are linked with increased health.[308,309] In addition to direct and indirect effects, there are also complex interrelated relationships between education and every socioecological aspect of human life.[304] For example, education often influences where people live, who constitutes their social and professional networks, and, reciprocally, mental and physical health influence the likelihood of attaining and succeeding in higher education.[304] All these effects make it difficult to tease out which specific aspect of education is responsible for the CVD risk reduction observed. This suggests a need for strategies to increase high school access and retention, especially for populations that are known to be at higher cardiometabolic risk and to be less likely to complete high school. This idea is detailed in the policy recommendations of section 7.5.

7.3.1.3. *Prenatal intervention to limit intrauterine exposure to diabetes as a hedge against offspring cardiometabolic risk*

Contrary to our *a priori* hypothesis, clinical trials that tested the normalization of glycemia in mothers with hyperglycemia (also termed mild GDM) did not prevent obesity in offspring when followed into childhood. Within the theoretical framework of the DOHaD theory,

interventions delivered in the first 1000 days of life (from conception to ~2 years of age) might help set a trajectory for health across the lifespan.[310–312] Within the area of maternal diabetes and offspring health, the most widely studied interventions concern the management of maternal glycemic during pregnancy.[313] As described in chapter 6, systematic reviews suggest that management of hyperglycemia in pregnancy reduces the risk of maternal (c-section, preeclampsia[270]) and infant complications (neonatal hypoglycemia, macrosomia, shoulder dystocia[212,213]). However, they also consistently highlight that high-quality evidence is scarce and that benefits come at the cost of higher rates of preterm birth, pharmacology therapy initiation, and healthcare visits.[270,314,315] In this context, we examined the impact of intensive glycemic management of mild GDM on health outcomes in offspring beyond infancy. Specifically, we assessed the effect of interventions designed to normalize maternal glycemia in pregnancy on childhood obesity, an important risk factor for early CVD.[274] Our systematic review of randomised trials enabled us to evaluate the potentially cardioprotective impacts of the implementation of new GDM diagnostic criteria on offspring while assessing the causal role of the intervention in this impact. While these trials were successful in reducing birth weight in offspring, we did not identify a statistically significant effect on obesity nor waist circumference in childhood. Other relevant outcomes of our review (fat mass, body mass index) were not reported and thus could not be analysed. This area of DOHaD research investigating how to increase health in offspring is therefore underdeveloped and could provide important complementary information to the population-based observations made in chapter 4.

Prevention of childhood obesity following intensive glycemic control during hyperglycemic pregnancies had been theorized by researchers and clinicians based on the continuous relationship between maternal glycemia and birth weight[235,237,316], and the

relationship between higher birth weight and childhood excess weight.[311,317,318] In reviewing the literature, it appeared that only a few studies – three to be exact – tested this hypothesis, each with non-significant results (Figure 6.2). The lack of significant effect of intensive glycemic management in women with mild GDM on childhood obesity might have been due to a limited normalisation of the intrauterine diabetes exposure. In fact, although GDM (and pre-existing diabetes) is diagnosed through measures of hyperglycemia, this condition is also associated with disruptions of circulating lipids, hormones, and inflammatory factors that affect the intrauterine milieu.[319–321] Hence, it is possible that treating hyperglycemia alone without normalising other aspects of maternal metabolism is insufficient to fully prevent cardiometabolic risk conditioning in the offspring. Alternatively, as women in the control group also exhibited only mild hyperglycemia during pregnancy, the difference in intrauterine exposure between intervention groups might not have been very large. This would explain the similar risk for excess weight in the offspring of both groups. This observation is supported by one of the trials which reported no difference in average glycemia between the treated and untreated group.[263] We conclude that even if offspring birth weight increases linearly with increasing levels of maternal glycemia during pregnancy, experimental data do not support the adoption of a universal normalisation of glycemia during mild GDM to reduce offspring obesity beyond infancy.

Our study reinforces the concept that observational data are often not sufficient to guide clinical and policy decision-making, as experimental results often reduce or contradict conclusions based on observational data. Our findings have important implications considering that a diagnosis of GDM has very tangible effects on mothers' familial relationships, psychological health, and on their concern for their baby or their own physical health.[322,323] Additionally, a recent analysis of individual participant data from 58 studies (n = 10 353) showed

that universal GDM screening was not cost-effective, irrespective of the screening approach used (net monetary benefit varying between −1197£ and −1210£ assuming 20 000£/quality-adjusted life-year).[235] The authors noted that they could not include long-term offspring outcomes in their models due to the lack of reporting of these outcomes in both observational and experimental studies. According to our results from chapter 6, including offspring obesity risk would not have improved their estimates. Another cost-effectiveness including long-term offspring outcomes, if and when these are reported, would be relevant to do as it might modify the estimates.

Thus, as intrauterine exposure to GDM has significant impacts on offspring CVD risk, an effective CVD prevention strategy from a public health perspective might be to focus resources on high-risk pregnant women and intervene more broadly in the postnatal period.

7.3.2. Sex differences in cardiovascular risk after intrauterine exposure to diabetes

Three of the four studies presented in this dissertation provide insight into potential sex differences in the relationship between intrauterine diabetes exposure and CVD risk. While sex did not significantly interact with high school completion to influence CVD risk in chapter 5 (Table 5.4), females displayed a slightly increased cardiovascular risk in the first two studies. In the analysis of adolescents with T2D in chapter 3, the sample consisted of a majority of females (65%) because it reflected the composition of the pediatric endocrinology clinic where 62% of patients with T2D are female.[324] Although we could not directly test for sex differences in that study due to the already restricted sample size, the sex difference in the sample is worth noting. Moreover, data collected in nationally representative samples in the United States showed that while rates of CVD-related deaths in males with diabetes were halved between 1986 and 2000

(from 26.4 to 12.8 annual deaths per 1000 persons), corresponding rates did not significantly change for females living with diabetes (from 10.5 to 9.4), suggesting a sex difference in the causes or treatments of CVD.[325] In that regard, intrauterine exposure to diabetes in youth living with T2D was possibly too small of an additional risk factor compared to the large risk attributable to female sex and existing T2D to see a difference in LV function.

In the population-based study presented in chapter 4, sex was a significant predictor for the composite cardiovascular outcome which included CVD risk factors (such as T2D and hypertension), but not for CVD endpoints (which excluded risk factors). This observation is in line with the argument above as it points to a relationship between intrauterine diabetes exposure, female sex, and risk of hypertension and T2D – the two main components of the main composite outcome at 61% and 25% respectively. In the general population, hypertension prevalence is similar in males and females, either in early adulthood (9% and 7% respectively at ages 20-34 years[326]) or across the lifespan (age-adjusted prevalence 31% and 32% respectively[273,326]). Considering our results, this might indicate that while males are generally considered at high risk of CVD, females also are at high risk of hypertension. This is concerning because hypertension is the foremost contributor to CVD mortality in females but not in males[204,273,327], suggesting higher CVD morbidity in females for a similar hypertension prevalence.

Sex differences have also been identified in animal models investigating cardiovascular risk following intrauterine exposures. Whereas fetal deprivation (restrictions in proteins, calories, or placental blood flow) increased males' risk for hypertension[57,328], such associations have not always been found in female offspring.[329,330] It was posited that females might be more sensitive to nutrient-dense exposures in light of studies showing that female rats exposed to

overnutrition (lard-enriched diet, high fructose) *in utero* had significantly elevated blood pressure and dysglycemia.[331] However, a recent systematic review and meta-analysis of maternal high-fat diet and offspring outcomes found that worse metabolic markers were more likely to be found in male offspring while highlighting that few studies tried to explain this sex difference.[67] It is not clear whether the disproportion of studies reporting outcomes in males (n = 70) *vs* females (n = 43) accounts for the discrepant results.[67,204]

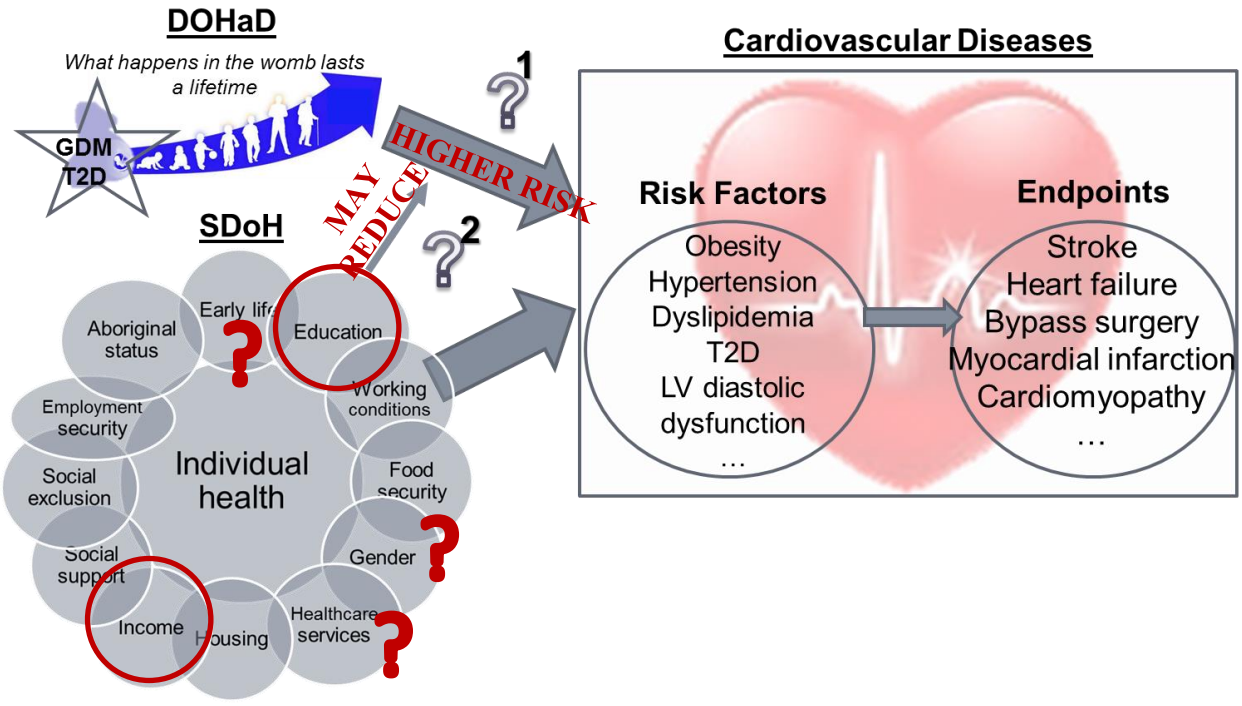
In summary, although it was not a primary focus of this dissertation, the evidence presented indicates that CVD risk attributable to intrauterine diabetes exposure might be different between sexes and this might impact the progression to CVD endpoints. This adds to the currently emerging recognition that CVD also affects females, especially in the context of intrauterine exposure to diabetes.

7.4. Integration of Findings with the Theoretical Framework

The findings from this dissertation have been integrated into the theoretical framework introduced in section 2.2. As we expected, our four studies inform this framework in a complementary manner. We can answer the first question mark (Figure 7.1) using the evidence presented in chapters 3, 4 and 6. This evidence suggests that intrauterine exposure to diabetes, either as GDM or pre-existing T2D, is associated with a higher and earlier incidence of CVD risk in offspring; this association is stronger for variables that increase the risk for CVD than for CVD endpoints in adolescence and early adulthood; and there is a dose effect indicating that milder, shorter forms of diabetes exposure (such as exposure to mild GDM) have lesser effects on CVD risk whereas more severe or longer forms (such as GDM and T2D) have worse effects

(younger age at first event, higher incidence of CVD endpoints). The words "higher risk" have been added to the arrow associated with the first question mark of figure 7.1 to reflect these conclusions.

Figure 7.1 Theoretical framework updated with the results of the dissertation



The blue "life course" arrow was used and modified with permission from the United States Developmental Origins of Health and Disease Society and the other images are public domain. The theoretical framework underlying this dissertation and originally presented in section 2.2 was updated with the words and symbols in red to reflect what was learned throughout this dissertation. In response to Question Mark #1: Exposure to GDM or pre-existing T2D is associated with a higher prevalence of CVD risk factors and endpoints. In response to Question Mark #2: Some social determinants of health may reduce the higher risk of CVD observed after intrauterine exposure to diabetes. More specifically, Education and Income were circled as high school completion and higher socioeconomic status were associated with lesser CVD risk after intrauterine diabetes exposure. On the contrary, neither our Early life marker (breastfeeding initiation) nor our Healthcare services marker (GDM diagnostic criterion change) had significant impacts, hence the question marks on these circles. Indeed, while our chosen markers were not significant, other markers in these categories might be important. Our results concerning sex differences suggest that gender might modify the relationship between intrauterine diabetes exposure and CVD risk, but this will need to be explicitly tested in future studies.

The second question mark on the theoretical framework is informed by chapters 5 and 6. This evidence suggests that certain social determinants of health may modify the association between intrauterine exposure to diabetes and CVD risk, but the effect depends on both the severity of the exposure and on the specific determinant investigated. High school completion, a specific marker related to "education" in the framework, had a significantly protective effect on risk of CVD in those exposed to GDM or T2D, contrary to breastfeeding initiation after birth, a specific marker related to "early life," in the same population. Interventions testing a modification of GDM diagnostic criteria, which relates to a modification of "health care services," did not result in a significant risk reduction in offspring exposed to mild GDM. The words "may reduce" were added on the arrow below the second question mark in Figure 7.1 to reflect the conclusion that certain social determinants of health may be associated with reduced CVD risk after intrauterine exposure to diabetes. The sex differences we observed or did not observe in all four studies indicate that gender might modify the relationship between intrauterine diabetes exposure, but this will need to be explicitly tested in future studies. It is relevant to highlight that other markers exist for the social determinants of health investigated in this thesis and could be studied as well. For example, the Early Years determinant encompasses many factors involved in intrauterine and post-partum development ("the first 1000 days of life"[310–312]) outside of breastfeeding including parental health and well-being, pollution exposure, and neurostimulation. Breastfeeding initiation is a limited marker of the Early Years determinant and should be interpreted strictly in the context of the study presented in Chapter 5.

Advantages of this framework, as mentioned in section 2, included highlighting the importance of intrauterine exposures in shaping early CVD risk, supporting multi-pronged policy initiatives to reduce this risk, and adding social determinants of health to the list of possible

factors intervening between intrauterine diabetes exposure and early CVD risk. This is in agreement with the work of Heckman (2000 Nobel laureate in Economy) and colleagues who highlighted how early-life investments in human capital yield the highest benefits.[332] Likewise, intervening on early CVD risk through policies and programs that change the structural environment within which populations live has the potential to make a bigger impact than current individual-based treatment strategies. While healthy lifestyles should be encouraged for everyone, policies can be used to facilitate these lifestyles rather than making them depend on people's circumstances. The next section will present some ideas that could be used early in life, be part of multi-pronged initiatives, and leverage social determinants of health to decrease the risk of early CVD.

7.5. Policy Recommendations

The conclusions from this dissertation are important for clinicians and policymakers as they suggest that screening/monitoring programs using intrauterine diabetes exposure as a risk factor and focusing on detecting hypertension and T2D might be effective in identifying adolescents at risk of early CVD. Although more research is needed to identify effective interventions to reduce CVD risk after this exposure, our results indicate we can prevent or at least delay progression to CVD endpoints in these young people.

7.5.1. Screening Recommendations

Currently, intrauterine diabetes exposure is not recognized as a risk factor for CVD. Widely used predictive scores and screening tools such as the Framingham Risk Score estimate

CVD risk in the short/medium term based on factors contemporary to the assessment and are validated only in adults ≥30 years old as they have not proven adequate in predicting CVD endpoints in younger adults.[272] This is partly due to a lack of recommendations for universal screening in this generally low-risk population.[272] The present dissertation suggests that in Manitoba at least, offspring exposed to diabetes *in utero* could constitute a non-negligible pool of individuals at increased risk of developing T2D and hypertension in their adolescence or early adulthood, which sets them on a high-risk path towards early CVD endpoints. New clinical guidelines could ensure that young people exposed to diabetes *in utero* are followed up more closely by their healthcare providers to recognize and prevent the development of T2D and hypertension. As these were the two main risk factors we identified, preventing or delaying their incidence would eliminate up to 86% of the CVD outcomes we investigated. Many health agencies refrain from recommending universal CVD risk screening in childhood due to financial, logistical, and ethical questions.[333,334] Indeed, no CVD screening tools were found that were applicable under 30 years of age. Based on our results, intrauterine diabetes exposure identifies a sub-population in which it is justified to screen for CVD risk in childhood.[333,334] Future studies should assess the contribution of intrauterine diabetes exposure compared to other known risk factors (such as familial and medical history) to confirm its usefulness in a clinical screening tool as well as the best age to start screening for CVD risk. Based on our results, screening should start before puberty as hypertension and T2D were already evident in the teenage years.

7.5.2. Recommendations to reduce CVD risk

The study presented in chapter 5 uncovered a strong association between high school completion and CVD risk after intrauterine diabetes exposure. Notably, high school completion

had similar protective effects in offspring exposed and not exposed to diabetes *in utero*. This observation supports the idea that ensuring high rates of high school completion could contribute meaningfully to reduced incidence and prevalence of early CVD in all young adults. Specifically, interventions and policy modifications to improve completion rates should be focused on populations that are more likely to suffer from chronic diseases and are less likely to complete high school. For example, Indigenous youth in Manitoba are four times more likely to develop early-onset T2D[179] and are less likely to complete high school (47.6%) compared to non-Indigenous peers (86.2%).[220] Worryingly, their school system is also chronically underfunded, and Indigenous youth must often attend school outside of their home community, which is a significant barrier to attendance and completion.[335] Our results suggest a link between these situations and support a revision of the education system to improve both education and health outcomes in these youths. The new First Nations School System[336], if properly funded and supported by the province of Manitoba, has the potential to reverse this disparity. Indeed, by tailoring the provision of education to participating Nations' cultures and ways of knowing, the First Nations School System will likely improve the retention and completion rates of its pupils. Tracking the education and health outcomes of these pupils might provide further evidence and incentives to solidify and expand this system to all the Indigenous Nations that wish to partake in it. In parallel, the province of Manitoba should prioritize investments in Indigenous youth education considering the strong association it has with CVD risk and the existing disparity in high school completion between Indigenous and non-Indigenous youth. Approaches effective in improving completion rates have the potential to reduce both educational and health-related disparities. Our results also suggest that in general, Education and Health offices should design and evaluate policies jointly to optimize government resources and population-level benefits.

7.5.3. Unintended negative impacts

While the results presented in this dissertation will need to be replicated to confirm the impact of intrauterine exposure to diabetes on early CVD risk, it is necessary to mention potential unintended negative impacts following their release. For example, insurance companies might change their scoring systems to include this exposure as an official risk factor for early CVD before its validation, which would negatively impact thousands of young Canadians. As we illustrated how early CVD risk following intrauterine diabetes exposure was also associated with sex differences and socioeconomic status, such changes in insurance policies could widen existing health disparities. Governments might need to create new legislation or policies to prevent such practices from insurance companies or to balance the resulting disparity. It will be necessary to critically evaluate these results and futures studies in this area to prospectively identify unintended consequences of our findings and try to mitigate them.

7.6. Limitations and Future Research

Some knowledge gaps remain as a result of limitations to the studies presented in this dissertation. A small number of observations has limited our ability to conclude with certainty about the impact of intrauterine exposure to diabetes on LV function and structure among youth living with T2D. Due to limited data from birth into adolescence and a relatively small sample size, we were also not able to explain our finding of smaller hearts in youth with T2D that were exposed to diabetes *in utero* when compared to those not exposed. *In utero* assessments of

cardiac function and development as well as animal models will be helpful in determining the timeline of cardiac development disruptions that lead to smaller hearts in adolescence following intrauterine exposure to diabetes. In addition, experimental studies using animal models of intrauterine exposure to diabetes will be able to assess the mechanisms involved in these disruptions, which our observational study could not do.

The observational design used for the studies in chapters 4 and 5 also hindered our ability to find causal relationships between intrauterine exposure to diabetes, intervening factors, and resulting CVD risk. Replication in other population-based cohorts and animal models will be needed to confirm our findings. Although we used GDM and T2D as dichotomous exposure variables, we are aware that intrauterine exposure to diabetes is a spectrum of exposure to rising glycemia, lipids, inflammation, and dysregulated adipokines. In this regard, future research should account for diabetes treatment or management during the index pregnancies, which we could not do due to lack of reliable data. The combination of diagnoses for CVD endpoints and CVD risk factors hampered our ability to distinguish the differential effects of intrauterine exposure to diabetes on these two sets of outcomes as well as on specific endpoints (such as myocardial infarctions only). Although this decision was made based on the limited number of offspring who received any of our specified diagnoses, future studies will be needed to assess the risk for endpoints and risk factors separately, to eventually untangle whether intrauterine diabetes exposure has endpoint-specific effects. This might mean waiting for the offspring from our cohort to grow up and develop CVD endpoints, or using data from other cohorts or registries if they contain reliable assessments of both intrauterine diabetes exposure and CVD endpoints in sufficient numbers. Nonetheless, our sensitivity analyses separating CVD endpoints and CVD risk factors support our main conclusions; future research might refine our findings rather than

contradict them. Again, we are not able to postulate on specific mechanisms that explain the association – or lack thereof – of high school completion and breastfeeding on CVD risk. As with all observational designs, our results may be sensitive to unmeasured confounding. Specifically, we could not assess the impact of family history of CVD, child excess weight/adiposity, nor of intrauterine exposure to maternal hypertension, which has been recently proposed as a potential confounder for child hypertension. However, this last exposure should not bias our current results too much. Current evidence from the population-based, high quality Generation R cohort indicate that intrauterine exposure to gestational hypertension is at most mildly correlated with offspring diastolic blood pressure at ~6 years old after adjustments (0.13 difference in standard deviation score, 95%CI 0.05-0.21; n = 5103 [337]) and preeclampsia was not significant in that study. Similar effects seen for paternal blood pressure and hypertension suggest that this relationship may be driven by genetic or familial factors rather than intrauterine exposure [337,338].

More research is needed on the effects of breastfeeding in offspring exposed to diabetes to confirm its non-significant (or protective) role towards cardiometabolic health. There is a dearth of research on the long-term impacts of breastfeeding on these exposed offspring, so it is not clear whether our results are the exception or the rule. In any case, our results should not be used to deter mothers with diabetes from initiating breastfeeding; the lack of protection towards CVD risk in early adulthood does not take away from the numerous known benefits of breastfeeding for the infant (reviewed in [339]).

Furthermore, we did not find that changing GDM diagnostic criteria did not reduce the risk of offspring childhood obesity. As with any systematic review, our results were limited to available trials and therefore should be interpreted strictly in that regard. In addition to

limitations mentioned in section 6.3, the systematic review design limited our capacity to harmonize the methods (interventions chosen, outcomes measured) between the studies, to account for missing data if it was not reported in the manuscript and the authors did not supply them, and to extrapolate our findings. The benefits of normalizing even mild hyperglycemia during pregnancy have been shown on relevant outcomes such as macrosomia and cesarean section, and our null results are not intended to challenge this.

New questions raised by our findings concern the cost-effectiveness of using a more inclusive diagnostic criterion for mild GDM, which is not likely to be positive if there are no additional long-term benefits for the offspring, and the inclusion of patients' voice when modifying universal screening and diagnostic criteria. Future research should focus on the follow up of offspring exposed to glycemic control intervention in pregnancy to try to elucidate potential long-term metabolic effects of these interventions on the offspring. In the meantime, our results propose there is more urgency in managing T2D in women of childbearing age than in managing mild GDM.

In Canada, patient-oriented research is gaining traction as a preferred method to answer the research questions that are relevant to patients and to bridge the gap between research, clinical practice, and policy.[340] Women with GDM or T2D have expressed that pregnancy is a pivotal moment in their and their child's life, that a GDM diagnosis is far from trivial, and that the medical benefits of current GDM diagnostic and treatment may not always surpass its disadvantages.[211,243,315,323] In this context and in light of our results, we recommend that patient-oriented approaches be used both in the development of research questions around GDM and in the development of clinical practice guidelines that help empower women to manage their own reproductive and overall health.

Finally, our findings suggest there might be a sex difference in the conditioning of, or the progression to, CVD in offspring exposed to diabetes *in utero*. In addition to the limited research on the CVD risk related to exposure to GDM and pre-existing T2D, the lack of reporting of sex-specific outcomes prevents the assessment and characterisation of sex differences in these conditions. Future studies should not only consider sex as a variable in their analyses but also report sex-stratified results to facilitate the identification of disease mechanisms that are shared by both sexes and those that may be different. Females should, therefore, be screened for CVD just as frequently as males as they do not appear to be protected from hypertension even in their premenopausal period.

7.7. Conclusions

As intrauterine diabetes exposure and CVD are becoming more prevalent in the Canadian population, this dissertation constitutes a novel contribution to inform better prevention of early CVD in the next generations. This dissertation found that intrauterine diabetes exposure is associated with an early CVD risk and that two factors may protect against this risk: high school completion and lower levels of socioeconomic deprivation. The novel evidence brought forward in this dissertation associates intrauterine diabetes exposure with a higher risk for CVD independent from other known risk factors and suggests that high school completion holds more potential for CVD risk reduction than other factors investigated (breastfeeding initiation, intensive glycemic management in mild GDM). We did not observe an association between exposure to diabetes *in utero* and LV structure or function in youth living with T2D. However, we did find an increased risk of hypertension in youth living with T2D and in the general population after intrauterine exposure to diabetes. Together, these four studies are the first to

investigate if intrauterine exposure to diabetes is independently associated with increased CVD risk in humans while controlling for many relevant confounders, and to investigate potential avenues to reduce diabetes exposure-related CVD risk at the societal level using factors that could be targeted using public health initiatives (in opposition to biological or lifestyle factors that rely on individuals).

By providing information on intrauterine diabetes exposure and social determinants of health as potentially modifiable risk factors for CVD in the next generations, our results contribute to the three following areas: they provide further evidence on the need to provide adequate and timely care to pregnant women diagnosed with, or at risk of, diabetes to reduce this harmful exposure in the next generations; they support public health initiatives focused on social determinants of health such as education as they can be modified by policy and impact entire systems (such as the Healthcare system and the Education system) to improve health on a large scale; and they inform future research on the relationship between intrauterine diabetes exposure and children's cardiovascular health.

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