THE IMPACT OF MATERNAL DIABETES IN PREGNANCY ON THE DEVELOPING CARDIOVASCULAR SYSTEM OF THE CHILD

by

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Abstract

Introduction

There is increasing evidence that fetal exposures influence lifelong cardiovascular (CV) health. While gestational diabetes mellitus (GDM) is one such exposure, whether there are demonstrable changes in CV health in exposed children is unknown. Additionally, rodent models have suggested maternal diabetes during pregnancy is associated with increased risk of myocardial ischemia-reperfusion injury (IRI) in adult offspring; a finding not explored in humans. The objective of our primary study was to explore the CV health of children of mothers with GDM (CGDM) compared to children of healthy mothers, also examining the additive impact of other prenatal and postnatal exposures. The objective of our secondary study was to determine whether infants of mothers with diabetes (IDM) have worse outcomes associated with cardiopulmonary bypass for major congenital heart disease compared to infants of healthy mothers, and whether this relates to worse IRI.

Methods

Primary Study: This was a nested observational study, tied to the Alberta Pregnancy Outcomes and Nutrition Birth Cohort Study. Children of mothers with and without GDM but otherwise uncomplicated pregnancies were prospectively recruited. CV outcomes included: left ventricular (LV) mass z score, reactive hyperemia index score (lnRHI, EndoPAT), Doppler-based aortic and peripheral arterial pulse wave velocity (PWV), and carotid intima-media thickness. Maternal dietary intake and iron biomarkers in pregnancy and the child's perinatal history were obtained, and the child's current anthropometric parameters were measured. Children completed three 24-

hour food recalls, and a Healthy Eating Index (HEI) score was calculated. Children's physical activity was measured using an accelerometer, and daily time spent in moderate to vigorous physical activity (MVPA) was calculated. Associations between these factors and CV outcome measures were explored with univariate and multiple linear regression models.

Secondary Study: IDM and infants of healthy mothers (controls) who underwent bypass surgery at <1 year at the Stollery Children's Hospital were retrospectively identified. Risk Adjustment for Congenital Heart Surgery (RACHS) scale was used for surgical coding and pooled into Groups 1-3 (A) and 4-6 (B). Primary (postoperative intensive care and hospital lengths of stay, LOS) and secondary (Pediatric Risk of Mortality (PRISM) score, lowest mixed venous O_2 saturation, highest lactate, glucose, urea and creatinine, and days intubated) outcomes were compared.

Results

Primary Study: We assessed 25 CGDM and 27 controls (mean age 10.9±0.7 years) with comparable birth/baseline characteristics. Aortic PWV was higher in CGDM vs controls (6.2±2.6 vs 4.8±1.6 m/s, p=0.03), and higher in CGDM of insulin vs diet controlled mothers (7.9±2.6 vs 5.5±2.2 m/s, p=0.03). No other primary CV health measures differed. Mothers with GDM, but not controls, demonstrated dietary improvement from the 2nd to 3rd trimester. The child's HEI scores and daily MVPA did not differ between groups. Univariate regression models revealed associations of both the child's percent body fat and HEI score with LV mass that persisted in a multiple regression model with inclusion of relevant covariates (B=-0.026, p=0.003 and B=0.020, p=0.008 respectively). Maternal serum ferritin in second (B=-0.0039, p=0.01) and third

trimesters (B=-0.011, p=0.008) negatively correlated with the child's lnRHI score in the univariate analyses, though the multiple regression models were inconclusive.

Secondary Study: Eighty IDM (54 gestational, 26 pregestational) and 149 controls were included: 188 Group A, 41 Group B. Age at surgery, birth weight and male:female did not differ between IDM and controls; whereas, maternal age (IDM vs Controls: 33±6 vs 30±6 years, p<0.001), gestational age at birth (37±2 vs 38±2 weeks, p<0.001) and delivery mode (49% vs 34% C-section, p=0.03) were different. Within RACHS groupings, outcomes did not differ between all IDM and controls. However, IDM-gestational showed trends towards better outcomes after surgery (Group A intensive care LOS: 3±3 vs 4±3 days, p=0.02; Group B highest glucose: 13.4±2.0 vs 16.7±3.0 mmol/L, p=0.01), while IDM-pregestational exhibited worse outcomes in Group A (hospital LOS: 20±29 vs 10±7 days, p=0.046; highest urea: 11.0±4.4 vs 8.4±4.2 mmol/L, p=0.04) and similar trends in Group B (PRISM: 16±9 vs 10±5, P=0.11; highest glucose: 16.7±1.3 vs 14.3±2.7 mmol/L, p=0.06).

Conclusions

CGDM have increased aortic stiffness, highest in mothers requiring insulin. Elevated maternal iron status may impact the child's endothelial function. Mechanisms and long-term implications of these findings require further investigation. Finally, the poorer outcomes associated with pregestational diabetes and better outcomes associated with GDM require further study.

Preface

This thesis represents original work by Cleighton Boehme, completed with the support and guidance of his supervisors, Dr. Lisa K. Hornberger and Dr. Rhonda C. Bell, as well as graduate committee members, Dr. Stephane Bourque, and Dr. Michael Khoury. The studies included in this thesis were formally approved through the University of Alberta's Research Ethics Board prior to commencing. The primary study of this thesis, entitled **"The Impact of Gestational Diabetes on the Cardiovascular Health of the Child"**, received ethics approval from both the Health Research Ethics Board - Health Panel at the University of Alberta (Pro00102292) and the University of Calgary Conjoint Health Research Ethics Board (REB20-1627). The secondary study, **"Surgical Outcomes in Infants with Major Congenital Heart Disease Exposed to Maternal Diabetes in Utero"**, received ethics approval under the title "Impact of Adverse Early Life Exposures on Surgical Outcomes of Congenital Heart Disease" (Pro00082478). Chapter 3, **"The Impact of Gestational Diabetes on the Cardiovascular Health of the Child**" (Authors: Cleighton Boehme, Martha Esparza Jimenez Moran, Victor Do, Rhonda Bell, Carminda Lamboglia, Elnaz Vaghef Mehrabani, Deborah Fruitman, Yazid Al Hamarneh, Ben Vandermeer, John C Spence, Christy-Lynn Cooke, Lisa K Hornberger), and Chapter 4, **"Surgical Outcomes in Infants with Major Congenital Heart Disease Exposed to Maternal Diabetes in Utero"** (Authors: Cleighton Boehme, Daniel Garros, Jimmy Kang, Amanda Cao, Dominic Cave, and Lisa K Hornberger), form the basis of original manuscripts which ultimately will be submitted for peer-reviewed publication over the coming months.

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There were many collaborators and others who made this research possible. Dr. Lisa K. Hornberger and Dr. Rhonda C. Bell provided guidance and direction throughout the projects. Furthermore, the work in the primary study of this thesis would not have been possible without the aid of the Alberta Pregnancy Outcomes and Nutrition (APrON) study team and participation of the APrON study participants. Andrea Deane, APrON Project Director, facilitated this work along with data analyst Henry Ntanda who worked diligently to facilitate data acquisition. Playing a major role in preceding work to lay the foundation for the primary study, assisting with grant writing, and staying involved through planning, collection, and interpretation of study data, pediatric resident Dr. Victor Do was instrumental throughout this project. Pediatric cardiologist Dr. Deborah Fruitman at the University of Calgary facilitated the work and ethics approvals for the participants who lived in Calgary. Pediatric cardiologist Dr. Michael Giuffre and the staff at Providence Pediatric Cardiology in Calgary, particularly Colleen Bole, clinic manager, and Swetang Desai, lead sonographer, facilitated use of the necessary space and equipment to

complete cardiovascular and anthropometric assessments in Calgary when this was not possible at the Alberta Children's Hospital due to COVID-19 safety considerations. ActiGraph Accelerometers were both purchased by the study team and provided by Dr. John C Spence, professor of kinesiology. Graduate students Carminda Lamboglia and Ashley McCurdy were instrumental in initializing ActiGraph devices and extracting data, with data validation completed by Carminda at the completion of the study. Nutritional data were analyzed by Dr. Elnaz Vaghef Mehrabani, a post-doctoral fellow at the University of Calgary and registered dietician, under the direction of Dr. Rhonda C. Bell. Dr. Martha Esparza Jimenez Moran, a senior post-doctoral fellow in pediatric cardiology, completed all echocardiographic assessments and assisted with the offline analysis of echocardiograms. Graduate student Juliana Lasso Mendez and Dr. Victor Do also helped with this analysis. Technical support throughout image acquisition and transfer was provided by Victor Aleksejev, a biomedical equipment technologist at the University of Alberta, with additional support from echocardiography technicians Warren Thompson and Brendan Haughian. Finally, EndoPAT training and support was provided by Rae Foshaug, the research coordinator in pediatric cardiology, and Dafna Sheer from Itamar Medical.

Others contributed to the work completed in the secondary study of this thesis. Dr. Daniel Garros, a pediatric intensivist at the Stollery Children's hospital, provided data and guided the research team through the process of acquiring additional Stollery Cardiac Intensive Care Unit data as needed. Lydia Fong, Cathy Cur, and Melissa Gardiner also assisted with data acquisition and transfer. Amanda Cao and Jimmy Kang initially began the project, looking at different adverse exposures, including preterm birth and fetal growth restriction, and helped establish a base on which to build this study examining surgical outcomes in infants with congenital heart disease after exposure to maternal diabetes.

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List of Abbreviations

1hPG: 1-hour plasma glucose

2hPG: 2-hour plasma glucose

aIMT: aortic intima-media thickness

ANOVA: Analysis of Variance

APrON: Alberta Pregnancy Outcomes and Nutrition

aPWV: peripheral pulse wave velocity

ASA24: Automated Self-Administered 24-hour Dietary Assessment Tool

BMI: body mass index

CFG: Canada Food Guide

CGDM: children of mothers who had gestational diabetes

CHD: congenital heart disease

cIMT: carotid intima-media thickness

CPB: cardiopulmonary bypass

CV: cardiovascular

CVD: cardiovascular disease

DOHaD: Developmental Origins of Health and Disease

ECFC: endothelial colony forming cells

EKG: electrocardiogram

ET: ejection time

FGR: fetal growth restriction

FOXO1: forkhead box protein 1

FPG: fasting plasma glucose

GDM: gestational diabetes

GE: General Electric

HAPO: Hyperglycemia and Adverse Pregnancy Outcomes Study

HEI: Healthy Eating Index

ICU: intensive care unit

IDM: infants of mothers who had diabetes in pregnancy

IGT: impaired glucose tolerance

IMT: intima-media thickness

IRI: ischemia-reperfusion injury

IVCT: isovolumic contraction time

IVRT: isovolumic relaxation time

IVSd: end-diastolic interventricular septal thickness

lnRHI: logarithm transformed reactive hyperemia index

LOS: length of stay

LV: left ventricle

LVOT: left ventricular outflow tract

LVPWd: end-diastolic left ventricular posterior wall thickness

MV: mitral valve

MVPA: moderate to vigorous physical activity

NCI: National Cancer Institute

NIH: National Institutes of Health

OGTT: oral glucose tolerance test

PAT: peripheral arterial tonometry

pPWV: peripheral arterial pulse wave velocity

PRISM: Pediatric Risk of Mortality

PWV: pulse wave velocity

RACHS: Risk Adjustment for Congenital Heart Surgery

RADIEL: The Finnish Gestational Diabetes Prevention Study

RHI: reactive hyperemia index

SGA: small for gestational age

TIBC: total iron binding capacity

Chapter 1: Introduction

Cardiovascular disease (CVD) is the leading cause of death, accounting for roughly one-third of deaths globally [1]. The World Health Organization attributes most of these deaths to suboptimal health behaviours, including poor diet, sedentary lifestyle, and smoking—thus, potentially preventable. Yet, there is increasing data in the literature suggesting some adverse exposures early in life, and even before birth, such as the presence of maternal diabetes, may contribute to an increased risk of CVD in the offspring during adulthood. Identifying such exposures, the mechanisms whereby they alter the cardiovascular (CV) system of the fetus and infant, and the timing and patterns of evolution of CVD are critical if we are to curb this devastating health issue.

The global prevalence of diabetes is roughly 10% and rising [2]. It has been understood for decades that diabetes has a significant adverse effect on CV health, contributing importantly to CVD in adults, with a greater impact on women [3–7]. In this introduction, we will share evidence of the effect of diabetes not only on the health of the individual, but also on the cardiometabolic health of the child in the context of exposure to the intrauterine milieu associated with maternal diabetes. The focus of this thesis is to explore the impact of diabetes in pregnancy on the CV health of the child, and to examine factors that may contribute to or mitigate risks. The ultimate goal is to better understand how to reduce the risk of CVD and support long-term health of these children from early stages. We begin with an overview of CVD and its aetiologies, not only in adulthood but also before birth.

1.1. CVD Overview

CVD is broadly defined as any structural or functional disease of the heart and blood vessels. While it includes conditions such as congenital heart defects (CHD), cardiomyopathies, arrhythmias, and rheumatic heart disease, a large proportion of affected individuals globally, particularly adults, have atherosclerotic CVD [8]. Atherosclerotic CVD involves narrowing of the arteries due to a buildup of plaque on arterial walls, originating in childhood, exacerbated by CV risk factors in early life such as high cholesterol, smoking, diabetes, and obesity, and progressing throughout life [9,10]. Atherosclerosis can be present in coronary arteries (coronary artery disease) which contributes to ischemia and myocardial infarction. It can also be present in carotid arteries and in the cerebrovascular bed and contribute to cerebrovascular disease and stroke. In addition, peripheral arterial disease can lead to reduced blood flow and subsequent pain, numbness or weakness, and sores, most often involving the lower extremities. Diffuse peripheral artery disease [11], as well as long-standing changes in vascular health that include increased arterial stiffness [12] and endothelial dysfunction [13], additional features of CVD, are intimately tied to the evolution of systemic hypertension, which, in turn, has a negative consequence on cardiac structure and function [14]. Excess weight, high blood pressure, and elevated low density lipoprotein and total blood cholesterol, which may be due to diet and/or a genetic predisposition, are well-established risk factors of atherosclerotic CVD, and many approaches are used to target these risk factors, including diet and exercise programs, blood pressure medications, and cholesterol-lowering statins. Inflammation and oxidative stress are also known to play important roles in the progression of atherosclerotic CVD, with many new therapies in development to specifically target pathological oxidation processes [15].

CVD is a significant problem in North America. One in ten adults in the United States has atherosclerotic CVD [16], with approximately 7% of adults above the age of 20 years having coronary artery disease [17]. As the risk of developing atherosclerotic CVD increases substantially with age, the proportion of affected adults in their mid and later decades is likely substantially higher. The prevalence of atherosclerotic CVD in Canada has recently been reported in a population study to be approximately 8% [18]. Adult CVD in North America comes at a substantial financial burden. In 2016 and 2017, direct and indirect costs to care for the population with CVD in the United States were estimated to total over $360 billion US dollars annually, or roughly 1000 USD per capita [17]. Comparable costs related to CVD have been found in Canada, with total annual costs of 21 billion CAD, or roughly 700 CAD per capita, representing approximately 12% of the total Canadian cost of illness [19]. Clearly, efforts to prevent CVD are worthwhile, with earlier prevention carrying the greatest promise in terms of savings in health costs and promoting lifelong health and well-being. Knowledge of the aetiologies responsible for adult CVD is not only key for effective treatment, but also, most importantly, for prevention.

1.2. Aetiologies of CVD

The Framingham Heart Study [20,21], a well-orchestrated, longitudinal, transgenerational population study initiated in the United States in 1948 following a rise in deaths from coronary artery disease in the preceding decades, led to leaps in understanding of the risk factors of CVD. Specific risk factors including high blood pressure, high cholesterol, smoking, and diabetes mellitus were identified in this landmark work. These factors along with others are now commonly used in health systems, including in Canada [22], to predict the risk of CVD using the "Framingham Risk Score". The Framingham Heart Study went on to include three generations of

participants, reflecting the inter- and trans-generational impact of CVD and CVD risk factors. Of note, women in the Framingham study represented more than half of the original cohort at a time when women were often underrepresented in health research. Adding to findings from the Framingham study, the Bogalusa Heart [10] and Pathobiological Determinants of Atherosclerosis in Youth [9] studies have established that CVD begins in childhood and progresses throughout life, highlighting the importance of establishing healthy behaviours early on.

Over the past three decades, there has been increasing evidence that, in addition to the risk factors previously discussed, adverse exposures even before birth may increase risks of CVD in offspring, also known as the Developmental Origins of Health and Disease (DOHaD) hypothesis.

1.3. Developmental Origins/Fetal and Early Life CV Programming Overview

The DOHaD hypothesis states that there are critical periods in fetal and infant development during which influences from the external environment can shape organs and organ systems to set the short- and long-term health trajectories of individuals. In regard to CVD, this concept has been termed “cardiovascular programming”. Seminal work published by David Barker and others in the 1980's and early 1990's began to illuminate the link between early adverse exposures and later CV health, with data obtained from detailed birth records in England and Wales beginning in 1911 [23,24]. Early findings from this work indicated that children born in geographical regions that had high infant [25] and maternal [26] mortality were at a higher risk of CV mortality in adulthood. They also found higher ischemic heart disease mortality in adults born of lower-than-normal birth weight [27], an exposure that was also linked to later development of diabetes [28], a risk factor for CVD. Furthermore, they found that both men and women were more

susceptible to CVD mortality when born of lower birth weight, with men being more adversely affected by sustained low weight through infancy [29]. This provided early evidence of sex-differences in the DOHaD model.

In addition to this early work by Barker, there have been many studies of the effects of famine during pregnancy that have also contributed to understanding of the relationship between maternal nutrition in pregnancy, fetal growth, and later health. The Dutch Hunger Winter represents one set of such studies [30]. Near the end of World War II, in 1944-45, food supplies were severely limited in Western regions of the Netherlands secondary to food transport blockades and subsequent freezing over of waterways due to harsh winter weather. For about six months, the daily calorie rations per person ranged from 400 to 800. This cohort represented a group of people who were well-nourished prior to the famine and whose diets returned to normal quickly after the famine, providing an opportunity to study the effects of severe dietary restriction in pregnancy on outcomes of the offspring. Researchers found that intrauterine exposure to famine, which would have also included other adverse exposures (e.g., stress, poor living conditions, etc.), correlated with adverse health outcomes in the offspring, with differential effects depending on the timing of exposure and the critical windows of development on various organ systems. In particular, famine exposure was associated with higher risk of CVD and of metabolic syndrome later in life [30]. Other famine studies around the world have supported these associations [31,32]. While many such studies have implicated maternal malnutrition and hampered fetal growth in the development of adverse long-term outcomes, not all studies have reached this conclusion. For example, studies of the Leningrad Siege famine found that maternal undernutrition in pregnancy did not contribute to later glucose intolerance or CVD risk at age 50 [33] nor was there a higher incidence of diabetes or CV events at age 70 in those exposed [34]. As

another example, a study of the Channel Islands Occupation [35] found that birthweight was not associated with CVD events later in life. Rather, exposure to the occupation, which was not associated with birthweight, predicted CVD later in life, indicating that exposures other than prenatal growth, such as undernutrition early in life or stress, may have been more important. Another study of the Channel Islands Occupation found that men exposed to the occupation in infancy and childhood, but not prenatally, had higher plasma glucose concentrations, indicative of adverse metabolic outcomes [36].

Altogether, these observations have led to an immense area of research focused on understanding how various adverse early life (fetal through infancy and childhood) exposures set individuals on lifelong health trajectories, for better or for worse. Such exposures include placental pathology with consequent fetal hypoxia, poor nutrition, and fetal growth restriction (FGR), famines and maternal dietary restriction, preterm birth, stress and emotional/psychological wellness, and maternal medical conditions affecting fetal growth and development, such as diabetes.

1.3.1. Mechanisms of CV Programming

In a recent review, Bourque & Davidge [37] described mechanisms implicated in CV health and disease following fetal programming. They defined these as instigator and effector mechanisms. Instigator mechanisms include system-wide processes, such as reactive oxygen species, epigenetic alterations, the renin angiotensin system, and the microbiome, which influence the development of organs. Effector mechanisms, on the other hand, represent changes to specific organs and organ systems that result in altered development and function throughout the life course. These include cardiac and vascular changes as well as changes in other systems

such as the kidneys, hypothalamic-pituitary-adrenal axis, and autonomic nervous system. Since its inception, CV programming has evolved into an extensive area of research, and many animal models have been developed to understand these mechanisms. These animal studies, along with observational studies in humans, have begun to paint a broader picture of this concept. The aim of the following sections is to explore evidence of how adverse in utero exposures affect the heart and vasculature of the offspring and to link this to intrauterine exposure to diabetes.

1.3.2. Low Birth Weight, Growth Restriction, and CV health

Data from human and animal studies with low birth weight and/or FGR further support this concept of CV programming and increased CVD risk [38]. This may be related to changes in both vascular and cardiac structure and function. These associations are explored in more detail in the subsequent sections.

1.3.2.1. Classifications of Low Birth Weight, Small for Gestational Age, and Fetal Growth Restriction

There are a number of different indicators of fetal growth that have been used. Low birth weight, small for gestational age (SGA), and FGR have been defined by the World Health Organization in the International Classification of Diseases 11th Revision [39]. The simplest of these is the classification of low birth weight, which includes any birth weights <2500 grams. SGA includes any infant born 2 standard deviations below the mean for their gestational age, or at <10th percentile. FGR is present when a fetus does not reach its predicted genetic potential or shows signs of malnutrition. These conditions are related, not completely dependent on one another. For example, a preterm infant can be born with low birthweight but not SGA or with

FGR. Additionally, an infant can be born SGA but not FGR and vice versa. These distinctions are important when considering the implications of study findings where differences indices of fetal growth are employed.

1.3.2.2. Low Birth Weight or FGR and Vascular Structure and Function

Adverse early exposures leading to vascular changes may predispose individuals to an increased risk of CVD later in life. As an example, low birth weight has been associated with higher blood pressure in adulthood, an important risk factor for CVD, with growth trajectories after birth also having an important effect, and these effects may be due to alterations in underlying vascular structure and function [40]. In particular, those who are of lower weight at birth and undergo accelerated weight gain in childhood have been shown to have a higher systolic blood pressure in adulthood [41–43].

There is evidence that changes in vasculature precede clinical changes in blood pressure [44], providing an early physiological basis for later disease risk. Low birth weight and FGR have been associated with increased aortic intima-media thickness (aIMT), a strong risk factor for early atherosclerosis [45–48], in newborns [49–51] and at 18 months [50,52,53]. Epure et al. [54] conducted a systematic review to examine associations between carotid intima-medial thickness (cIMT) and exposures from conception to two years of life, including gestational age at birth, weight, length, and head circumference, mode of conception, maternal diabetes, and smoking in pregnancy, and found the most consistent association to be in infants born SGA. Crispi et al. [55] also demonstrated increased cIMT in 5-year-old children with FGR/born SGA, as well as increased systolic and diastolic blood pressure. Increased cIMT represents thickening of the artery wall and is indicative of the progression of atherosclerosis and risk of CV events later in life [56]. Increased

arterial stiffness suggestive of alterations in vascular structure in preterm very low birth weight infants has also been observed [57], and there is some suggestion that increased arterial stiffness may persist until later childhood in children born with low birth weight [58]. In addition, endothelial dysfunction may also be present in later childhood following low birth weight [58], contributing further to the pathogenesis of hypertension and CVD.

Further work in animal models has provided evidence to elucidate the mechanisms involved in these vascular alterations. In a protein restricted rat model of FGR, Menendez-Castro et al. [59] found increased expression of connective tissue growth factor in the aortas of newborns as well as both increased myocardial collagens and collagen I in the aortas of 70-day old rats, in FGR as compared to controls. Additionally, a rat model of uteroplacental insufficiency found increased stiffness, endothelial dysfunction, and alterations in the collagen composition of uterine arteries in growth restricted female offspring [60]. Changes in expression of these collagens is linked to differences in vascular structure and function, with a reduction in the distensibility of the vessels and subsequent hemodynamic alterations. These subtle changes in the structure of the vascular wall are likely linked to the pathogenesis of CVD. Furthermore, increased arterial stiffness and the accompanying increase in afterload may contribute to adverse myocardial remodeling which could culminate ultimately in altered cardiac function [61].

Additional work has been done in animal models to understand the effects of FGR on later endothelial function as well as the mechanisms involved, including the role of oxidative stress [62]. It has been shown that nitric oxide mediated vasodilation via the endothelium is inhibited in adulthood in placental insufficiency and hypoxia-induced FGR models in male [63,64] and female [65] rats, with a possible increased susceptibility at an earlier age in females [65]. Finally, another study using a similar model of hypoxia in rats found that adult male FGR rat offspring

were hypertensive and had alterations in their vascular endothelin-1 system, whereas females did not [66]. Together, these studies highlight a sex-difference in affected mechanisms and provide additional evidence of the CV programming effect in FGR and low birth weight.

1.3.2.3. Low Birth Weight or FGR and Cardiac Structure and Function

FGR has also been shown to have long-term effects on the developing heart. Sarvari et al. [67] found changes in cardiac structure and function associated with FGR in preadolescents. In particular, they found that the hearts of preadolescents who had experienced FGR were smaller and more spherical than those who were born without FGR, a finding that aligns with other investigations [68–71]. They further observed their hearts exhibited reduced longitudinal strain as well as compromised diastolic dysfunction and postulated that these changes may cause the heart to be less efficient, with important long-term consequences [67]. Whether the effects represent a direct insult on the myocardium or indirect from altered vascular health is not clear.

Data from animal studies provides further support. In a study using a hypoxia model of FGR in rats, Rueda-Clausen et al. [72] found that later in life, offspring exposed to in utero hypoxia had left ventricular (LV) diastolic dysfunction and increased mass as well as pulmonary hypertension. They also found a sex difference, with males being more susceptible with greater changes in LV mass [72]. The same research team showed increased ischemia-reperfusion injury (IRI) following short periods of ischemia suggesting the reserve, coronary or otherwise, may be reduced [73]. Recent work at the University of Alberta using the same fetal hypoxia rodent model has suggested that although myocardial functional changes, specifically diastolic dysfunction, evolve in early life (1-2 weeks), in fact measurable increases in arterial stiffness may start even earlier, even from postnatal day 1, and likely contribute to these myocardial changes [74].

Myocardial changes following FGR have been shown on the cellular level from fetal stages to adulthood. Animal models of nutrient restriction, uteroplacental vessel ligation, and hypoxia-induced FGR have shown a reduction in the number of cardiomyocytes along with cellular hypertrophy in affected offspring in the late fetal and neonatal stages [75–77]. Rat models of FGR, both protein restriction and hypoxia induced, have shown an altered expression, deposition, and remodeling of collagens in the neonatal period and adulthood and fibrosis in adulthood in hearts of affected offspring [59,78,79]. In addition, rabbit models have shown impaired energy metabolism and mitochondrial arrangement relative to myofilaments in fetuses [80] and decreased sarcomere length in adulthood [81] indicating underlying substrate for reduced functional capacity in cardiomyocytes following FGR.

1.3.3. Other Exposures and Programming of Cardiometabolic Risk

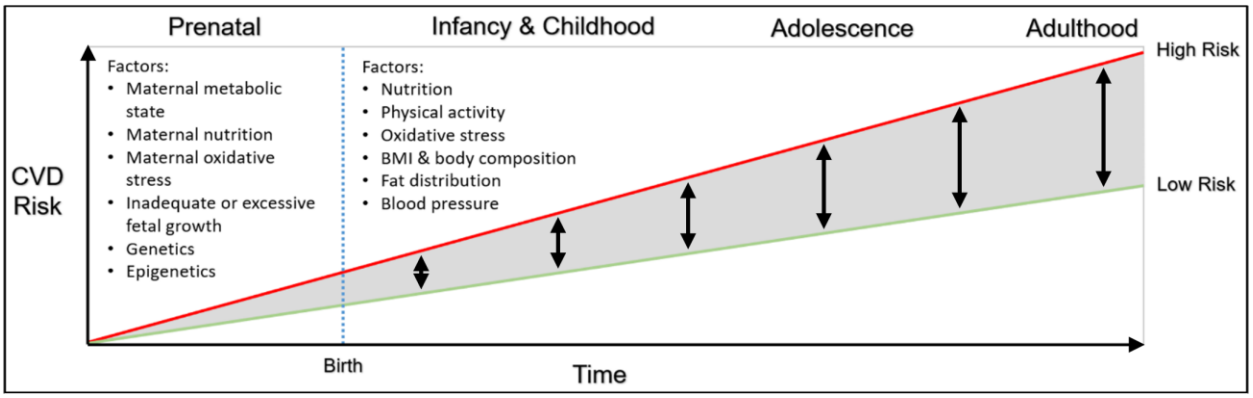
In addition to the adverse effects of undernutrition and FGR, other factors can contribute to CV programming. Preterm birth and stress [82] as well as older maternal age [83] are other factors increasingly recognized as negatively impacting the long-term CV health of children. Moreover, others have identified maternal overnutrition and obesity in pregnancy to lead to adverse cardiometabolic health in the offspring, including higher blood pressure, adverse lipid profiles, and evidence of glucose intolerance, all risk factors for adult CVD [84]. Fetal overgrowth and macrosomia may also lead to adverse CV and metabolic outcomes. Recent results from the Cardiovascular Risk in Young Finns Study [85] revealed that infants born large for gestational age (birthweight >90th percentile) were more likely to be obese and had increased cIMT in adulthood, which carry longer term CVD risks.

Although maternal, placental, and neonatal exposures clearly impact cardiometabolic health of the child, clinical studies have shed further light on the role of postnatal factors as well as biological sex. In a systematic review examining clinical studies, Ludwig-Walz et al. [86] found that offspring anthropometry rather than maternal pre-pregnancy body mass index (BMI) or weight better explained offspring systolic blood pressure. Of the 15 studies examining the link between maternal BMI or weight and offspring systolic blood pressure included in this review, 5 included adequate adjustment for maternal age, smoking, and socio-economic status and children's age and sex. Though these 5 studies found maternal BMI or weight to be associated with the offspring's systolic blood pressure, only one remained significant when adjusting for measures of the child's anthropometry. This highlights the importance of the postnatal environment and the difficulty of differentiating the direct effects of the in-utero environment from possible genetic or other health behaviour exposures. Interestingly, Kaseva et al. [87] found that increased insulin resistance and an adverse lipid profile in offspring, markers of cardiometabolic risk, were correlated with exposure to gestational diabetes (GDM), with differences that persisted after adjustment for important covariates, including the offspring's adiposity. Separately, they found that excess pre-pregnancy maternal weight (BMI≥25), a risk factor for GDM, was associated with higher fasting glucose and insulin levels in the child, though these differences were attenuated with adjustment for relevant confounders [87]. Finally, in mothers with both GDM and a BMI≥25, they found a greater effect on offspring fasting glucose and insulin levels than with increased BMI alone, although, again, these differences were attenuated with adjustment for relevant covariates, largely explained by the offspring's current BMI [87]. Finally, a recently published review by Talbot & Dolinsky [88] explored the differential effects of maternal obesity and diabetes on men and women. They suggested there are epigenetic

processes in fetuses, triggered by adverse in utero exposures, which have differential impacts on males and females later in life, and that these effects may be modulated by the offspring's postnatal diet and health behaviour factors, such as their level of physical activity.

Clearly, there are many interacting factors that contribute to an individual's long-term health and risk of non-communicable disease such as CVD. Exposures before and after birth contribute to determining the risk of cardiometabolic disease. This notion has been termed the life-course model. Adverse in-utero exposures place people on life trajectories of health, with postnatal exposures and health behaviours modulating this risk after birth. Figure 1.1 provides a simplified representation of such prenatal exposures and postnatal modulating factors.

Figure 1.1. A Life Course Model of CVD Risk



Legend: Prenatal factors such as maternal metabolic state, nutrition, and oxidative stress, inadequate or excessive fetal growth, genetics, and epigenetic processes set individuals on lower or higher long-term CVD risk trajectories. Postnatal factors such as nutrition, physical activity, oxidative stress, body composition, and blood pressure also contribute to long-term CVD risk and can help individuals move from higher trajectories that originated before birth to lower risk trajectories or vice versa. Adapted from World Health Organization, 2002 [89]

1.4. Diabetes in Pregnancy

The discovery of insulin a century ago brought about the possibility of pregnancy for women with diabetes, and advances in medicine over the years have improved outcomes for these women and their children [90]. The latter are exposed to an intrauterine milieu characterized by maternal insulin and glucose disequilibrium, impaired beta cell function, elevated lipids, inflammation, and a host of other metabolic changes. With the global epidemic of obesity and diabetes, 1/6 births are now affected by maternal diabetes, 84% of which are GDM [91].

In addition to effects on the mothers, the effects of diabetes in pregnancy on the infant may extend well beyond pregnancy, impacting the life course of the offspring. It is well-recognized that offspring born to mothers with diabetes can experience high birth weight (defined as >4000 g or >4500 g) or low birth weight as a result of FGR [92] (the risks of which have already been explored) and have a higher risk of delivery complications, most notably of emergency caesarean sections. It has also been shown that the offspring of mothers with diabetes, both pregestational and gestational, are at a higher risk of obesity, impaired glucose tolerance, and diabetes themselves [93–97]. Cohort studies in India [98,99] and Hong Kong [100,101], focused on the impact of diabetes in pregnancy, support these associations.

Exploring these associations further, it is important to note some of the nuances. In the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study with 25,505 females, it was shown that the risk of adverse outcomes related to the degree of hyperglycemia observed, with an elevated risk of adverse outcomes even with glucose levels below diagnostic thresholds [102]. Furthermore, it has been suggested the risk of many long-term adverse outcomes may be determined less by the type of maternal diabetes and more on the degree of hyperglycemia [103]. Biological sex differences may also influence the cardiometabolic effects of diabetes in

pregnancy on the offspring. Tam et al. [94] found that while hyperglycemia in pregnancy was associated with increased BMI, impaired glucose tolerance, and blood pressure in children in the HAPO study—trends that were also confirmed in a systematic review [95]—maternal hyperglycemia was only associated with increased adiposity in girls at 7 years of age. There may also be a confounding effect of BMI in mothers who experienced diabetes in pregnancy on cardiometabolic outcomes in offspring. A study in the US found that though GDM was associated with both birth weight and higher BMI in adolescence, much of this association was explained by maternal BMI [104].

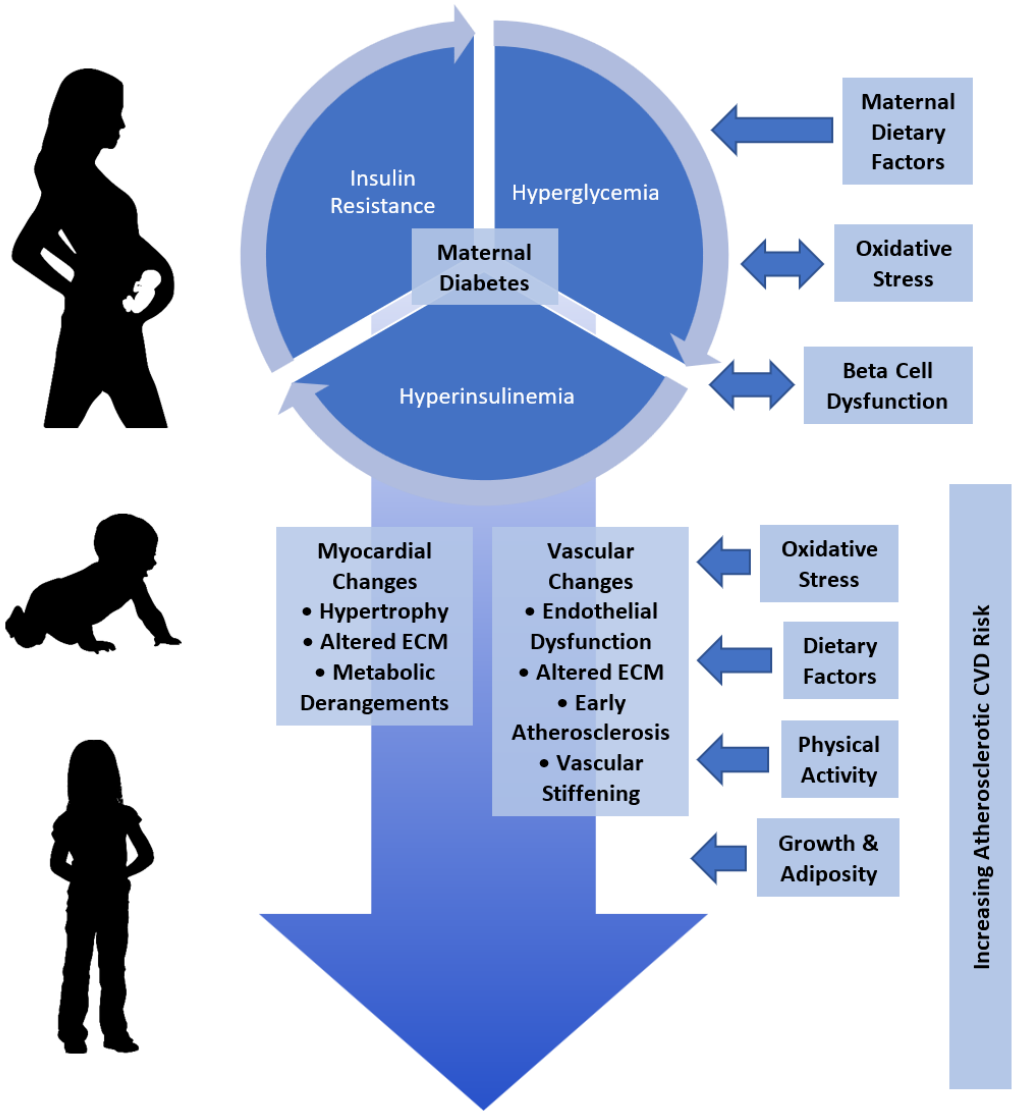
Additionally, although both postnatal health behaviours and genetic factors have been shown to contribute to increased risk of diabetes [105], convincing evidence from sibship studies in the Pima people, a Native American population in Arizona with a high population rate of diabetes [106], has suggested that in-utero exposure to diabetes further predisposes the offspring to increased risk [107]. In this study, it was shown that siblings born to the same mother after diagnosis of type 2 diabetes had higher risk of developing diabetes than those born before the development of maternal diabetes.

Though there are many interacting and confounding factors, there is convincing evidence to show that at least some of the adverse CV effects of maternal diabetes in offspring result from fetal exposure to hyperglycemia, with the level of risk related to the degree of hyperglycemia. As previously described, however, the diabetic milieu is much more complex than a simple elevation in glucose levels. Hyperglycemia, hyperinsulinemia and insulin resistance, and the associated inflammation and oxidative stress in pregnancies complicated by diabetes may all play important roles in determining maternal, fetal, and infant health.

1.5. Fetal CV Programming in Maternal Diabetes

Strong evidence from large population-based studies in Manitoba, Canada [108] and in Denmark [109] suggests that adult offspring of women with diabetes in pregnancy, both gestational and pregestational, have a higher risk and earlier onset of atherosclerotic CVD [110]. In another large population-based study in Israel, Leybovitz-Haleluya et al. [111] found that children of mothers with GDM were at an increased risk of CV-related hospitalizations in the first 18 years of life. Figure 1.2 provides an overview of proposed mechanisms contributing to increased CVD risk following pregnancies complicated by maternal diabetes. ***Though it is well-established that the offspring of women with GDM have an increased risk of CVD in adulthood, the mechanisms that contribute to this risk and the timing of development remain ill-defined.***

Figure 1.2. Mechanisms Contributing to CVD Risk in the Context of Maternal Diabetes



Legend: Maternal diabetes is characterized by a cycle of insulin resistance, hyperglycemia, and hyperinsulinemia. This can be caused by or contribute to beta cell dysfunction as well as systemic oxidative stress. Maternal diet also plays a critical role in the management of diabetes. Effects of diabetes in pregnancy on the cardiovascular health of the child that have been explored and are under investigation include myocardial and vascular changes. The child's oxidative stress, dietary factors, physical activity, growth, and adiposity may also contribute to their cardiovascular health, with increasing risk as the child ages. ECM: extracellular matrix

1.5.1. Maternal Diabetes and Cardiac Structure and Function

In addition to the risks and birth complications previously discussed, it has been well-established that diabetes in pregnancy has adverse effects on the developing fetal heart. Pregestational diabetes increases the risk of abnormal fetal development and subsequent birth defects, including CHD, with the risk increasing with the degree of hyperglycemia [112]. By the tenth week of pregnancy, the heart is fully formed, thus early exposure to hyperglycemia and the diabetic milieu, particularly in poorly controlled diabetes, imparts the greatest risk of CHD. In a Canadian study, Feig et al. [113] found that though the rate of diabetes in pregnancy doubled from 1996 to 2010, the rate of congenital anomalies from such pregnancies declined by 20-23% as did the rate of perinatal mortality in women seen by obstetricians, which may suggest improved glycemic control and consequent outcomes with close monitoring of such pregnancies.

Beyond the elevated risk of fetal CHD in pregnancies complicated by diabetes, it has also been well-established that diabetes in pregnancy, both pregestational and gestational, imparts subtle structural and functional changes to the fetal heart. This includes myocardial hypertrophy, particularly involving the interventricular septum [114–117], which persists at least into the early prenatal period [117], as well as altered diastolic function [118–121]. Although these abnormalities have previously been thought to regress and even resolve within the first months after birth, recent work at the University of Alberta suggests increased LV mass in the third trimester, may persist, at least for some, well into early childhood, [122]. While most of the clinical work has been in pregestational diabetes, fetuses of mothers with GDM have been shown by echocardiography to have more globular hearts and subclinical reduction in systolic function that persists into infancy, as well as reduced diastolic function in infancy [123].

Animal models have been used to further explore the consequence of maternal diabetes on cardiac structure and function after birth. Using a streptozotocin-induced diabetes rat model which leads to pancreatic beta cell destruction, Agoudemos et al. [124] found adult male offspring of affected dams had impaired diastolic function at 8-10 months of age, with decreased compliance of the LV. Abnormalities have also been demonstrated in a transgenic rodent model lacking the insulin receptor, with increased LV hypertrophy related to cardiomyocyte enlargement, altered cardiomyocyte gene expression, and decreased global and circumferential strain [125]. Interestingly, the differences reported were only present when the offspring were fed high-fat diets rather than normal chow in the post-weaning period [125], highlighting the important interplay between the pre- and postnatal environments in modulating the risk associated with in utero exposure to maternal diabetes. Taken together, these animal studies provide additional evidence of the effect of maternal diabetes on fetal and postnatal cardiac structure and function.

The mechanisms contributing to increased LV mass in late fetal stages through childhood and altered myocardial function following in-utero exposure to maternal diabetes are not fully understood. Maternal hyperglycemia causes excess glucose transfer to the fetus and elevated fetal insulin production. Rat models have shown organ specific expression of insulin-like growth factor and implicated its role in fetal myocardial hypertrophy in pregnancies complicated by diabetes [126,127]. However, beyond the possible direct effects of elevated insulin and insulin-like growth factor on myocardial hypertrophy in utero, vascular changes and elevated blood pressure in the offspring may contribute further to adverse cardiac structural and functional adaptations.

1.5.2. Maternal Diabetes and Vascular Health

Several investigations have demonstrated an association between exposure to diabetes in utero and increased blood pressure that may contribute to risk of long-term CVD and again negatively impact the myocardium. Cho et al. [128] found 12-year-old children of mothers with diabetes in pregnancy, gestational and pre-existing, to have higher systolic blood pressures than children of healthy mothers. In a systematic review including eight studies with over 7000 children in total that investigated the relationship between GDM and systolic blood pressure, Pathirana et al. [95] found children exposed to maternal GDM before birth demonstrated increased systolic blood pressures relative to those of healthy mothers. Prenatal exposure to GDM may also alter the child's response to stress which may in turn contribute to poor long-term CV health, potentially implicating the hypothalamic-pituitary-adrenal axis. A study with participants from the Mysore Parthenon birth cohort study in India found that adolescent children of diabetic mothers had an elevated cardiosympathetic stress response, including higher systolic blood pressure, as compared to children not exposed to diabetes in utero [129]. As population studies have demonstrated a direct relationship between elevated blood pressure in childhood and systemic hypertension in adulthood [130], with a continuous positive correlation of risk of vascular events with blood pressure [131], increased blood pressures observed in children of diabetic mothers is one mechanism whereby this prenatal exposure contributes to long-term CVD.

Beyond blood pressure, other aspects of vascular structure and function are important considerations in CV health, and alterations may in fact contribute to elevated blood pressure over time. Macrosomic infants born to women with diabetes have been shown to have increased abdominal aIMT as compared to both macrosomic and non-macrosomic infants born to mothers without diabetes [132]. Increased aortic/arterial stiffness and associated LV hypertrophy has also

been documented in late infancy among those exposed to pregestational diabetes, a finding that persists into childhood [122,133]. As altered arterial stiffness has been shown to correlate with adverse LV remodeling, including hypertrophy and fibrosis with consequence changes in function [134,135], this may represent another mechanism that contributes to altered long-term CV health among offspring of diabetic mothers. In the work of Do et al. [122], aortic pulse wave velocity, a measure of arterial stiffness, and not LV mass in these children correlated with maternal third trimester Hemoglobin A1c. As hemoglobin A1c measures glycemic control in the 3 months prior [136], this finding suggests a potentially critical time period of exposure to hyperglycemia that is relevant to the pregnancy complicated by GDM which evolves and is tested for in the second trimester. ***The impact of GDM and its degree of severity on the CV health of the child throughout childhood and adolescence, however, to date has not been fully explored.***

While diabetes in pregnancy has been shown to correlate with elevated blood pressure and vascular changes in the offspring, the mechanisms contributing to these alterations are not understood. Using an in vitro model, Ingram et al. [137] found that there were fewer endothelial colony forming cells (ECFC) in cord blood from pregnancies complicated by diabetes and that the ECFC that were present were less functional, even in cases of well-controlled diabetes. ECFC are important for the development of a healthy endothelium, the inner layer of the blood vessels that responds to and regulates changes in blood pressure [13]. Thus, fewer and less functional ECFC in infants whose mothers had diabetes in pregnancy may have impaired endothelial function from birth, and this may contribute to vascular changes and elevated blood pressure as the children age. Another study utilizing umbilical cord tissue following caesarean section deliveries in pregnancies complicated by diabetes found increased expression of genes

involved in vascular development and thicker umbilical veins and larger umbilical artery and venous areas relative to controls, suggesting possible pathways for altered vascular development in the offspring of mothers with diabetes in pregnancy [138].

Little work has been done in animal models to explore vascular changes and investigate possible mechanisms in the offspring of mothers whose pregnancies were complicated by diabetes. Although not explored in children, animal models have suggested renal dysfunction may be a contributing factor to higher blood pressure following prenatal exposure to a diabetic environment [139–141]. Furthermore, in a rat model, De Sa et al. [142] found that only adult male offspring exhibited vascular dysfunction and hypertension, again highlighting the importance of including biological sex as a factor in considering the effects of in utero exposures.

1.5.3. Recent and Ongoing Studies Investigating the Impact of GDM on the CV Systems of Offspring

In 2017, a new cohort study was proposed in Switzerland, the MySweetHeart Cohort study, to follow 100 GDM and 100 control mothers through their pregnancies and into early infancy and beyond to better understand the effects of GDM on the developing CV systems of children [143]. This project is still in the recruitment stage [144]. Another similar study in Finland made use of a pre-established cohort study, the Finnish Gestational Diabetes Prevention (RADIEL) study, to observe CV structure and function in six-year-old children. The RADIEL study included women with pre-pregnancy obesity as well as women with GDM in a previous pregnancy. They found that these children had increased cIMT compared to a reference Finnish population and that carotid arterial stiffness correlated with first and second trimester fasting glucose [145]. They also found that adiposity in the children correlated with the mothers' pre-

pregnancy adiposity [146,147]. This study was somewhat biased by the population included as they initially recruited only mothers at high risk of developing GDM and did not directly assess a more general population for comparison. Furthermore, although they collected dietary information on the mothers during pregnancy, they did not assess other postnatal health behaviours in the children. ***At this time there is a need for additional work comparing CV outcomes in children exposed to GDM with children of healthy women without and not at risk for GDM. The main study of this thesis will add to our understanding of how maternal GDM affects the CV health of the child.***

1.6. Additional Prenatal & Postnatal Factors that May Affect a Child's CV Health

Diet therapy is the first-line approach in the management of GDM [148]. Therefore, maternal diet is likely to have an important impact on the developing child. Moreover, the children's own nutrition patterns as well as levels of physical activity are important considerations in their CV health. These factors will all be explored in the main project of this thesis and are presented in more detail below.

1.6.1. Maternal Nutrition

The extent to which maternal diet quality and specific dietary factors impact the child's CV health in pregnancies complicated by diabetes in particular has not been fully explored. Highlighting the importance of maternal nutrition, Van Elten et al. [149] found in a systematic review that high overall carbohydrate intake in pregnancy correlated with higher systolic blood pressure in pre-school and school-aged offspring while negative association had been shown between maternal overall protein intake and offspring cIMT in school-aged children and young

adults. Normia et al. [150] also found higher blood pressure in 4-year-old children to be associated with higher maternal carbohydrate intake, but a moderate dietary fat intake in both mother and offspring corresponded with the most favourable blood pressures.

Maternal iron status in pregnancy may be an important micronutrient to explore among GDM pregnancies. Several investigations have established correlations between maternal iron status, iron intake (particularly heme iron), and increased risk for GDM [151–156]. Both too much and too little iron can have adverse effects on pregnancy outcomes and newborn health, highlighting the importance of finding an optimal balance [157]. In adults with high iron stores, reduced endothelial function has been observed [158,159], providing a basis for a possible association between high maternal iron in pregnancy and altered vascular health of the child.

1.6.2. The Child's Own Nutrition and Physical Activity

Both animal models and human studies have provided evidence that diet and physical activity can have important effects on the cardiometabolic health of children in general, potentially modulating the effects of other negative exposures. Appannah et al. [160] found that energy-dense, high fat, low fibre diets were associated with higher levels of insulin and insulin resistance in children, with increased waist circumference in females and increased fasting glucose and overall general metabolic risk, using an alternative classification system designed for children consistent with the metabolic syndrome [161], in boys. Turning to additional evidence from animal studies, Rueda-Clausen et al. [162] found that a high fat diet exacerbated the negative effects of FGR on the CV system in rat offspring.

With regards to healthy eating, Delgado-Floody et al. [163] found that adherence to a Mediterranean diet pattern was associated with lower systolic blood pressure in children.

Conversely, Saidj et al. [164] did not find any protective effects of diet in children exposed to suboptimal in utero conditions; however, they did find high levels of light physical activity to be protective. As for unhealthy exercise patterns, low levels of physical activity and low cardiorespiratory fitness levels have been associated with increased cardiometabolic risk. For example, stationary (i.e., sedentary) time has been associated with excess weight [165]. In addition, another study showed that baseline cardiorespiratory fitness level had an inverse association with systolic blood pressure and rate pressure product at two-year follow-up with changes in fitness levels also having an important effect on these measures [166]. Furthermore, a meta-analysis investigating the association between cardiorespiratory fitness and CVD risk found that both male and female children with low fitness had greater likelihood of CVD risk in adolescence, defined by the presence of CVD risk factors (e.g., elevated glucose, skinfold thicknesses, blood pressure) [167]. ***Clearly, the child's diet and exercise patterns are important, but the extent to which it can augment or attenuate any negative effects of maternal GDM is less clear.***

1.7. Overview of Thesis Research

To further explore the effects of diabetes in pregnancy on the CV health of the children, the current thesis comprises two separate studies. The primary study investigated the impact of GDM on the CV health of the child. With delays in commencing the primary prospective research due to COVID-19, a secondary chart review project was included in this thesis to further explore the effects of maternal diabetes on the CV health of children and to provide additional experience in clinical research. Specifically, this secondary study explored the associations between maternal diabetes in pregnancy and surgical outcomes for CHD requiring cardiopulmonary bypass in infancy, examining measures indicative of greater IRI.

1.7.1. GDM and CV Health of the Child

There are several important knowledge gaps to be addressed by the primary study of this thesis. While there is strong evidence that children of mothers who had GDM are at an increased risk of developing CVD later in life, the mechanisms and timing of development of this risk are not fully understood. There is little evidence comparing measures of CV structure and function in childhood and early adolescence following prenatal exposure to GDM as compared with uncomplicated pregnancies in a representative sample of the general population. Furthermore, the extent to which severity of GDM (diet versus insulin controlled) and other pre- and postnatal factors—including maternal diet and metabolic status and the child's own diet, metabolic status, and physical activity—modulate the effects of this exposure has not been fully explored.

In order to address these knowledge gaps, the current thesis sought to explore the effects of GDM on LV mass, aortic and peripheral arterial stiffness, endothelial function, and carotid intima-media thickness in children. Additionally, we explored relationships with other measures of cardiac structure, systolic and diastolic function, and vascular health. In the primary investigation, a prospective cross-sectional assessment of the CV health of preteens of mothers whose pregnancies were complicated by GDM was performed comparing findings in these children with age and sex-matched children of healthy mothers without GDM in pregnancy. This investigation recruited participants in the Alberta Pregnancy Outcomes and Nutrition (APrON) cohort [168], which provided prospectively collected nutritional data in pregnancy as well as data regarding postnatal exposures in the children, particularly regarding their diets, metabolic health, and activity levels.

This project had 3 study aims:

Aim 1: To compare the CV health of children of mothers who had GDM (and otherwise uncomplicated pregnancies) with the CV health of age- and sex-matched children from uncomplicated pregnancies and explore differences in offspring following diet and insulin managed GDM. *We hypothesized that children of mothers with GDM would have increased arterial stiffness and LV mass, with a pronounced effect in pregnancies requiring insulin therapy.*

Aim 2: To explore the relationship between maternal diet and iron status in GDM pregnancies and the CV health of the child, with data collected retrospectively through the APrON birth cohort. *We hypothesized poor maternal diet quality and high iron status would be associated with poorer CV health in the children.*

Aim 3: To investigate the impact of the child's dietary intake, metabolic status, and exercise on their own CV health. *We hypothesized worse diet and metabolic health (high cholesterol, insulin resistance), as well as physical inactivity, would be associated with worse CV health.*

As GDM is more common than pregestational diabetes, affecting as much as one in seven pregnancies [91], and as GDM is now on the rise in Canada [113], this research is targeting a large population providing answers to relevant knowledge gaps that may ultimately lead to strategies to improve the health of these children life-long.

1.7.2. Contribution of in Utero Diabetes Exposure to IRI

DOHaD studies have shown that gestational age at birth, birth weight, and maternal gestational weight gain influence surgical outcomes in infants with CHD [169–173]. Both FGR [172]

(diagnosed during pregnancy) and SGA [173] (diagnosed at birth) have been associated with worse postoperative outcomes. Furthermore, Asrani et al. [169] demonstrated that abnormal maternal gestational weight gain, either inadequate or excessive, also puts infants undergoing single ventricle palliation at increased risk of death and/or need for transplant. In pregnancies affected by maternal diabetes, both maternal gestational weight gain and fetal growth may be abnormal. Animal models have suggested adverse early life exposures including hypoxia before birth [73] and maternal diabetes [174], increase risk of myocardial injury following a period of ischemia with reperfusion, which is an insult that occurs with cardiopulmonary bypass used in cardiac surgery. It has been suggested following prenatal exposure to maternal diabetes, metabolic alterations, specifically impaired insulin signaling, may be primarily responsible for greater risk of IRI [174]. Despite these compelling findings in animal models, the impact of such adverse early life exposures including maternal diabetes on response to ischemia and reperfusion after birth in any age has never been clinically examined.

Cardiopulmonary bypass, in which the heart is arrested for cardiac surgery, is associated with evidence of IRI. This was first recognized in pediatric CHD in landmark work at the Hospital for Sick Children in Toronto in infants following surgery for tetralogy of Fallot [175–177]. This was also recognized in surgeries for transposition of the great arteries [178]. Although the hearts are both perfused with cardioplegia, a formulation designed to provide glucose and maintain ion balance [179], and cooled to reduce oxygen consumption, most demonstrate evidence of IRI within the first 1-2 days after surgery. More severe IRI may result in renal injury, delays in and inadequate diuresis, weaning from inotropes, and extubation, resulting in lengthier intensive care unit stays. In the worst responses, cardiac arrest may occur along with an ensuing need for greater support.

The aim of the second study of this thesis was to explore whether diabetes in pregnancy predisposes offspring with CHD to worse outcomes after cardiac surgery, possibly due to greater IRI, in humans. To this aim, we investigated the surgical outcomes of infants with CHD undergoing bypass surgery in the first year of life with an emphasis on their clinical picture within the first 1-2 postoperative days, when IRI is largely manifested. *We hypothesize that maternal diabetes in pregnancy represents an exposure that may predispose infants with CHD requiring early intervention to increased IRI and thus worse outcomes.*

1.7.3. Summary of Impact

As outlined throughout this introduction, diabetes in pregnancy represents a condition that predisposes the child of an affected mother to a higher risk of poor health outcomes over their lifetime. The proposed research initiatives will further elucidate the impact of this exposure on the general CV health of affected children and the role of postnatal exposures in contributing to or mitigating risks that may lead to long-term atherosclerotic CVD. Whether, at least in infancy, there is evidence of altered myocardial health resulting in poor response to ischemia will also be explored. This new knowledge will ultimately contribute to identifying strategies to improve the CV health of these children life-long.

Chapter 2: Methodology – Primary Study

2.1. Overview

The primary study in this thesis, investigating the impact of fetal exposure to gestational diabetes (GDM) on the cardiovascular (CV) health of children, has many different methodological considerations. These begin with the study design—a nested observational study tied to an established cohort study—and comprise examination of several exposures from prenatal stages through childhood, and the measures of CV health outcomes, including different vascular and echocardiographic-based ventricular function measures. This chapter will describe the various aspects of the study including important strengths and weaknesses of the chosen methods and practical considerations.

2.2. Study Design

2.2.1. Use of a Cohort Study

Cohort studies represent an excellent study design to investigate exposures at an earlier time point and to track outcomes. This can afford important data in human populations that may otherwise be inaccessible. Most cohort studies, however, are not of sufficient longevity to examine the impact of early exposures on longer-term outcomes, such as the current, and many can be quite costly. The primary research completed in this thesis represented a nested observational study, accessing participants and utilizing the wealth of data collected through the Alberta Pregnancy Outcomes and Nutrition (APrON) study, a large, prospective longitudinal cohort study. This particular cohort study included data collected at the beginning and throughout the study at specific time-points, facilitating comparisons between participants with

different exposures and reducing recall bias. The observational nature of cohort studies can make it difficult to eliminate confounding factors; these studies lack the precise control that is possible with experimental or intervention study designs. Despite this lack of control, prospective cohort studies make studying certain exposures possible that may be unethical to study experimentally, such as the impact of smoking on long-term health in humans. Data regarding early exposures in cohort studies is dictated by the original study design, and data regarding additional exposures of interest may be impossible to obtain retrospectively, necessitating the establishment of new cohort studies. Though this is unavoidable, this point highlights the significance and impact of the enormous amount of time and resources necessary to initiate and manage cohort studies. Additionally, there is the challenge of participant dropout over time in cohort studies, and great effort must be taken to keep participants engaged and interested in continuing participation.

2.2.2. A Matched Observational Study Tied to a Cohort

Given the complex nature of human health and the scarcity of certain conditions, identifying individuals with an exposure of interest and finding well-matched controls who did not have the exposure in observational studies can be very useful. Matching for selected characteristics is important to ensure that there are no additional important factors that may be influencing study results, particularly when there may be confounding factors. For example, obesity is a risk factor for diabetes. Investigating the impact of diabetes on long-term health may be confounded by the effects of obesity. Matching participants for body mass index (BMI) aids in reducing these confounding effects to investigate the effects of diabetes alone. Furthermore, in the case of this study, nesting within an established cohort study, which involved working with the APrON study team to identify mothers who were participating in the study and had been

diagnosed with GDM and then reaching out to them to ask if they would be willing to participate in our study, was useful to gain detailed data regarding early exposures and to prioritize the use of limited resources, particularly research funding and time restraints. Rather than relying on maternal recall of details during their pregnancy or the time-consuming review of medical records, both of which are limited in the scope and accuracy of data that can be collected, we had access to the wealth of information collected by the APrON study throughout the mother's pregnancies.

2.3. Recruiting from the APrON Study

2.3.1. Introduction to the APrON Study; Strengths and Limitations

The APrON study was established in Alberta, Canada in 2008 to investigate the effects of perinatal depression on neurodevelopmental outcomes in children. Further, the original investigators were curious to explore connections between maternal/prenatal nutrition, mental health, and the development of the child [180]. Initial questionnaires assessing maternal pre-pregnancy physical activity and dietary habits were collected and maternal diet, physical activity, and anthropometrics were assessed during each trimester of the pregnancies. Women recruited at or prior to 13 weeks gestation were assessed in each trimester while women recruited between 14 and 27 weeks were only assessed in the second and third trimesters. Follow-up rates in the study were 88% in the third trimester and 67% at 12 months post partum [180]. As described previously, retention of study participants in cohort studies can be challenging. In addition, mothers provided blood samples in each trimester. The following information was collected from women during pregnancy: medical histories, smoking, alcohol, and/or drug use, demographic information, and DNA samples. DNA samples were also obtained from the fathers and children. These variables

were all used to explore factors that influence maternal, as well as paternal, mental health, and their relationship with neurodevelopmental outcomes in the children.

In total, 2140 women, 2172 infants, and 1417 biological fathers were enrolled in the cohort [180]. All study participants were either from the Calgary (85%) or Edmonton (15%) areas [181]. The mothers were on average 31 years old, 96% were in married or common law relationships, 88% had completed postsecondary education, 78% had household incomes above $70 000, 77% were born in Canada, 80% were Caucasian, and 54% of the children were male [180]. The APrON team has previously reported that the cohort participants were older, were more likely to be primiparous, had higher rates of postsecondary education, and had higher income than the general population of pregnant women in Canada [182]. These differences may represent a limitation of the study, possibly reducing the generalizability of findings, but it also adds to the strength of the study in important ways. As APrON mothers had a high socioeconomic status, were highly educated, and had few barriers to health services throughout their pregnancies, they are likely to have faced fewer exposures to social and economic factors that can contribute to adverse pregnancy outcomes. None of the mothers enrolled in our study smoked during their pregnancies and they were generally physically active and well-nourished. Thus, they were an ideal cohort to investigate subtle changes in CV structure and function in the children following in utero exposure to GDM, with fewer confounding variables to consider.

For the main study of this thesis, investigating the impact of GDM on the CV health of the child, the researchers recruited children (8-12 years) and their mothers over a six-month period, including healthy children of mothers with GDM born at >36 weeks of gestation and age- and sex-matched healthy children born to healthy mothers without GDM and following an uncomplicated pregnancy. All children were recruited from the APrON birth cohort. The

children's gestational age at birth and biological sex as well as the mothers' age, pre-pregnancy BMI, weight gain during pregnancy, and parity were provided by the APrON study and used for matching controls to cases with GDM. In addition to these data, detailed information regarding maternal health history, lifestyle (including smoking, alcohol, and/or other drug use), and nutrition status during pregnancy was provided by the APrON study team. At the time of the current study, the children underwent CV (echocardiographic assessment of cardiac structure and function, aortic and peripheral artery stiffness and other measures of atherosclerosis, endothelial function, and blood pressure assessment) and metabolic (BMI, waist and mid upper arm circumference, and skinfold thickness) assessments. Information regarding the children's demographics, medical history, current nutrition, and physical activity was also collected. These data were used to explore the differences between the groups regarding their CV health, the contribution of the mothers' metabolic and nutrition status during the pregnancies, and the contribution of the children's own dietary habits and levels of physical activity.

2.3.2. Defining Case & Control Groups

2.3.2.1. Defining the Primary Exposure: GDM

GDM was first identified clinically in the 1950's [183]. Since that time, diagnostic criteria have gone through many permutations, and the understanding of the condition has evolved. In 1954, Hoet identified the increased risks to mothers and fetuses in pregnancies complicated by GDM [184], and then in 1957, an oral glucose tolerance test was first proposed for women at an increased risk of GDM or who had an abnormal screening test [185]. Since then, the diagnostic criteria for GDM have been widely debated with still no international consensus on the optimal screening method [186]. Current guidelines in Canada [187] recommend screening between 24 to 28

weeks, beginning with a 50-gram glucose challenge test for all pregnant women. If 1-hour plasma glucose is below 7.8 mmol/L, the pregnancy is considered normoglycemic. Any values from 7.8 to 11.0 warrant an additional oral glucose tolerance test (OGTT). If the value is ≥11.1mmol/L, GDM is diagnosed. The OGTT in Canada consists of a fasting plasma glucose (FPG) test followed by consumption of 75 grams of glucose with subsequent plasma glucose measures at 1h (1hPG) and 2h (2hPG). If one of the three following criteria are met, a diagnosis of GDM is made: FPG ≥ 5.3, 1hPG ≥ 10.6, or 2hPG ≥ 9.0 mmol/L. Current Canadian guidelines offer an alternative, 1-step approach to diagnosing GDM, consisting of a single 75 g OGTT with lower thresholds. This alternative approach is consistent with current guidelines from the International Association of the Diabetes and Pregnancy Study Groups, based on results from the Hyperglycemia and Adverse Pregnancy Outcomes study.

Considering the pregnancies of the primary study in this thesis took place between 2009 and 2012, it is important to consider Canadian diagnostic criteria for GDM at that time. Diabetes clinical practice guidelines released in 2008 also recommended a 2-step approach to diagnosis GDM, but the cut-off values were slightly different than the current values. The lower limit for an OGTT following a 50-gram challenge test was the same, but the upper limit warranting immediate diagnosis of GDM at the time was lower: 10.3 mmol/L. Further, though the thresholds following a 75-gram OGTT were similar (the same except for 2hPG levels: 8.9 mmol/L in 2008 versus 9.0 in 2018), 2008 guidelines required 2 abnormal values for a diagnosis of GDM, with one abnormal value leading to a diagnosis of impaired glucose tolerance (IGT) in pregnancy [188].

For the primary study of this thesis, self-reported GDM status from APrON surveys was used to identify participants to contact for recruitment. Attempts were made to contact all 68

eligible APrON participants who self-identified as having GDM, and 29 consented to participate, two of whom did not complete the in-person visit or other follow-up assessments. In one case, they indicated that they were too busy and in the other, the child did not wish to give their assent. Another mother/child dyad was later excluded from the analyses due to the child being born at 34 weeks gestation. Prior to completing the consent process, all participants were asked the same screening questions which included confirmation of GDM status during the APrON pregnancies. After recruitment was completed, the study team reviewed the participants' medical records to obtain results from glucose tests during their pregnancies. These data were used to verify GDM status. In fact, after reviewing medical records as well as APrON records, it was determined that one mother who self-identified as having GDM in fact had type 2 diabetes. This individual and her child were excluded from the analyses. Furthermore, the definition of GDM was expanded for the purposes of this study to include both those diagnosed with GDM and IGT in the GDM group. The inclusion of those diagnosed with IGT brings the GDM group in closer alignment with current diagnostic criteria in Canada. Twelve of the final 25 participants who self-reported having GDM, in effect, were identified as having IGT diagnosed in their pregnancies rather than GDM.

2.3.2.2. Selection of Children Whose Mothers Did Not Have Diabetes in Pregnancy

After children whose mothers had GDM had been recruited, the study team began reaching out to possible controls. Working with the APrON study database personnel, controls were matched for the children's age (within one year) and biological sex and the mothers' age at delivery (±3 years), pre-pregnancy BMI (underweight: <18.5; normal weight: 18.5 to <25.0; overweight: 25.0 to <30.0; or obese: ≥30.0) [189], weight gain during pregnancy (below, within, or

above Health Canada/Institute of Medicine gestational weight gain guidelines) [190], and parity (null: yes or no). Due to the difficulty of matching for many criteria, the criteria were given an order of priority. The child's biological sex and age were both matched without exception. As for maternal characteristics, maternal pre-pregnancy BMI and age were given priority for matching, while weight gain during pregnancy and parity were given the least priority. For the most part, mothers were able to be matched by age, but in a few cases, due to advanced maternal age, there were no available controls within three years of the mother's age. In these cases, the mother with the greatest age who was otherwise a good match was selected. If there were no suitable mothers within the same BMI category, their precise BMI was considered, and mothers were selected who had a close BMI, within one BMI category. Similarly, with the weight gain during pregnancy category, controls were identified within one category if not in the same category. As an additional note, after identifying three to five possible control participants for each case, control participants living in Edmonton were called first to save travel time for in-person assessments. In the end, the two groups were very comparable based on the matching characteristics.

2.4. Other Exposures of Interest: Lifestyle Factors

2.4.1. Maternal Diet

At each assessment during their pregnancies, women completed a 24-hour food recall, including food, drinks, and supplements, to provide a snapshot of their dietary intake during their pregnancy. Initially, research assistants with a background in nutrition conducted interviews to assess dietary intake, using a "multiple pass method" [191]. From August 2010 and on, after ~600 participants had completed food recalls with research assistants, the validated, web-based, Food

Behaviour Questionnaire [192–194] was employed. The format was similar to the multiple pass interview but used an automated system, including more than 800 foods.

There are some limitations of these dietary assessment methods to consider. Twenty-four-hour food recalls serve as an acceptable method of assessing dietary intake, though they may not be as accurate as food records kept throughout the day. Twenty-four-hour food recalls are less tedious and therefore more practical to adopt. Additionally, as described by Conway et al. [191], using a multiple pass method can lead to an overestimation of actual food intake, particularly in normal weight or overweight women, while women who were obese tended to recall their intake more accurately. As the majority of mothers in the APrON study were of normal weight or overweight (mean BMI: 24.1; standard deviation: 4.7) [180], the mothers' intakes may have been overestimated. However, since the mothers enrolled in the GDM and CV health of the child study were matched for pre-pregnancy BMI, this overestimation was likely comparable between groups and not likely to interfere with findings. Furthermore, the different methods of food recall data collection may also have influenced the results; however, since children in our GDM study were matched for age, this distinction may not affect the results either. Beyond these limitations, since 24-hour food recalls only include foods eaten in the last day, they may not accurately reflect individual habitual dietary intake, although when combined across participants, it does represent dietary intake at the group or population level. Some types of food frequency questionnaires may give a better picture of habitual dietary intake, but these can lack precision for estimating overall food intake. One strength of the APrON study is that it used both food frequency questionnaires to assess dietary intake prior to the pregnancy and 24-hour food recalls during the pregnancies [180].

2.4.1.1. Dietary Quality Score

To give a reflection of the mothers' dietary quality in the APrON study, a study team scored women's food intake [195] using the 2007 Canada's Food Guide (CFG) [196] as a reference to assess diet quality. Methods for scoring women's diet quality is described in detail by Jarman et al. [195]. Briefly, each participant was given a score out of nine that reflected whether they met (yes/no) the recommended intake in each category noted in CFG. Since the scores were for each food category were binary, they may not give a balanced representation of the women's adherence to the food guide. There were three points possible within the vegetables and fruit category, two for whole grains, two for dairy and alternatives, and one for meat and alternatives, with the final point for consuming additional servings recommended in pregnancy. This approach helps to facilitate reproducibility of the scores and gives insight into diet quality relative to recommendations at the time.

In addition to this score reflecting adherence to the 2007 CFG, the study team also scored women's diets according to their intake of less healthy foods and beverages such as soft drinks, chips, and cakes and cookies. Scoring intake of less healthy items allowed researchers to examine these 2 general categories (healthy and less healthy foods) separately. Taken together, it provides a very practical view of food intake, and moved beyond describing dietary intake by considering only energy, macronutrients, and micronutrients.

2.4.1.2. Iron Markers During Pregnancy

In each trimester of pregnancy, hemoglobin, serum ferritin, and total iron binding capacity (TIBC) were assessed from blood draws. Hemoglobin represents about two-thirds of total body iron [197], and iron-deficiency is a primary factor that has been associated with low

hemoglobin [198]. Therefore, hemoglobin levels can serve as an indicator for low iron, though low iron intake is not the only cause of anemia. Serum ferritin is another biomarker of iron status and reflects iron stores. A low levels of serum ferritin is indicative of low iron stores, although this can be masked by inflammatory conditions, as serum ferritin levels increase in the presence of inflammation [199]. TIBC, on the other hand, follows the opposite trend to serum ferritin. TIBC reflects the amount of iron that can be carried in the blood via transferrin proteins and is typically elevated with low iron status [200]. Low TIBC, however, is only present in iron depleted states; it does not indicate iron stores [201]. Considering each of these iron markers alone leaves room for interference from other conditions and may not give a complete picture of iron status, but using different markers in combination provides a more comprehensive picture of the different physiological pools of iron. Other markers of iron status such as hepcidin and erythropoietin and inflammatory markers such as c-reactive protein may have also helped to gain a more accurate picture of iron status, but data about these markers were not available from APrON participants at the time that this thesis was written.

2.4.2. Children's Lifestyle Factors

2.4.2.1. Children's Diet

Given the difficulty of accurately assessing dietary intake in children, the study team for this thesis asked children to complete three 24-hour food recalls, with assistance from their parent(s), around the time of their CV measures to assess their dietary intake. This method has been validated for use with children over the age of 8 years [202]. The research team used the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool version 2018-Canada, a web-based dietary intake collection tool [203]. This tool was designed by the United States'

National Institutes of Health's (NIH) National Cancer Institute (NCI), Division of Cancer Control & Population Studies and is available for use free of charge. It uses the multiple pass method previously described, and results have been shown to be comparable to interviewer administered recalls [204].

When the children came in for the in-person visits for the GDM study, the researcher (CB) reviewed the 24-hour food recall instructions with families. Families were instructed to complete three days of food recalls, including two weekdays and one weekend day. The researcher (CB) instructed the families to use a prepared food and drink logbook as a tool to remember foods eaten in order to report them on the ASA24 website the following day, with the children being assisted by the parents. The food and drink logbook included instructions on the front page and spaces for the dates, times of consumption, foods or drinks consumed, amounts, and locations.

Completion of multiple-day food records can be difficult and requires a significant time commitment. However, 3-day food records have been shown to correlate well with 9-day food records and provide an acceptable method of classifying individuals by nutritional intake [205]. Allowing families to select three days during the week permitted some flexibility to work the food recalls into their schedules. However, not all participants completed all of the recalls requested. As well, many participants did not complete the food records according to the instructions. Rather than entering the information the following day, they recorded the foods and then waited until a later date to enter the foods into the ASA24 website. Since the ASA24 website is designed to reflect food intake in the last 24 hours, recall dates collected in the ASA24 website did not consistently reflect dates that were recorded. Though the precise dates may not be crucial, the timing of record completion and recall entry may have affected the accuracy of

the recalls, as the food and drink logbooks may not have included sufficient detail to complete all required fields in the ASA24 recalls without the accurate recall of foods consumed the prior day. Allowing families to select days of the week to complete recalls may have also created a bias, as families may have been more likely to select days that gave a more favourable impression of their eating patterns.

Data from the food recalls were used to generate Healthy Eating Index scores [206] as described on the United States Department of Agriculture Food and Nutrition Service [207], using the NIH/NCI SAS code [208]. Furthermore, total energy and macronutrient intakes were generated for each participant and reported in the study results.

2.4.2.2. Children's Physical Activity

Data regarding the child's physical activity patterns were collected using an ActiGraph accelerometer to track activity for 7 days following the in-person visit. Completing activity tracking after the visits allowed the study team to demonstrate how to properly wear the ActiGraph and then hand it over to families to be worn for the week. Participants were instructed to wear the ActiGraph when awake and not in water. An activity log was also prepared to provide instructions to remind families of the proper use and wearing of the ActiGraphs and to collect information about the days of the weeks and dates the device was worn, the times the children woke up and went to bed, if they removed the device during the day, and any reasons it was removed. The activity logs were used to verify the data collected by the devices, including the days they were worn. Envelopes were provided for families to return the trackers and activity logs in the mail once they had completed the seven days.

Each child was instructed to wear the ActiGraph on the right side of their hip using an elastic waist band provided. The devices were initialized with a sampling frequency of 30 Hz, the device default, which has been shown to produce more accurate counts when the raw data is processed [209]. Further, the devices were set to record activity in 15 second epochs. Since a child's activity often happens in spurts, with frequent sporadic movements, shorter epochs provide a better resolution when observing their activity; therefore, using epochs of less than one minute in children and adolescents has been recommended [210]. Though different cut-off points have been proposed for the different levels of activity, Evenson's method [210], which uses 15 second epochs, has been shown to be more accurate than other methods [211], with cut-off points (per 15 second period) of 25 counts or less for sedentary or stationary activity, 26 to 573 counts for light, 574 to 1002 for moderate, and 1003 or greater for vigorous physical activity. Concerning wear time, a minimum of 10 hours was considered a valid day [212]. Non-wear time was defined as 45 minutes of consecutive, uninterrupted, zero counts [213]. In addition, 4-5 days of wear time has been determined to be the minimum acceptable amount of time, as this number of days can account for day-to-day variability in activity in children, achieving a reliability of 0.80 in children in grades 1-6 [214]. The research team for the present thesis study decided to set 5 days as the minimum requirement, as the children in this study were at the older end of this range and monitoring older children for longer periods of time has been recommended [214].

For tracking of physical activity, there are other considerations of importance. Though wearing an ActiGraph may make children more motivated to engage in physical activity, the choice of having the device be worn around the waist minimizes this risk. In addition, the device was plain and did not have a screen to display metrics or motivate the children to exercise. This feature of the ActiGraphs made them a desirable choice for this research over other devices.

Another important consideration is seasonal variations in physical activity patterns. A Danish study has shown that in the winter, children spend more time in sedentary activity and less time in moderate to vigorous physical activity [215]. The impact of this variation on the outcomes of the main study of this thesis was also minimized, as all data collection occurred within the period of a few months, during the winter and into the first week of spring.

2.5. Anthropometric Measures

During the in-person visits, the study team took several of the children's anthropometric measures, including height, weight, waist circumference, mid upper arm circumference, and triceps skinfold thickness. These measures were used to calculate BMI z scores, lean body mass, waist to height ratio, and percent body fat based on arm circumference and skinfold measurements.

2.5.1. Height, Weight, BMI, and Lean Body Mass

Children's height and weight were measured at the beginning of their assessments. They were instructed to remove their shoes and any additional clothing such as heavy sweaters or coats before being weighed. They kept their shoes off for height measurements. As age and sex have significant effects on BMI [216], BMI z scores were calculated based on the United States' Centers for Disease Control and Prevention reference charts, accounting for the children's age, height, and biological sex [217]. Although on the individual level, BMI measures do not take different body types into account, BMI has been shown to be strongly associated with body fat [216]. Another measure of body composition which has been shown to be especially important in

considering cardiac growth and development, lean body mass, was calculated with validated, sex-specific equations, using the child's height, weight, and BMI z score [218].

2.5.2. Circumferences and Skinfold Thickness

Waist circumference, mid upper arm circumference, and triceps skinfold thickness were all measured in the children. Waist circumference was measured with a non-elastic tape measure around the waist between the lower costal ribs and above the iliac crest, with the tape measure placed either directly on the skin or over light clothing. They were instructed to keep their hands by their sides and exhale before the measurement was recorded. Waist circumference and waist to height ratio have been shown to be better predictors of CV risk than BMI in children [219,220].

For mid upper arm measurements, the children were instructed to hold their elbow at 90 degrees with their forearm parallel to the ground and palm open, facing up. A mark was made midway between their acromion process and olecranon process, and the arm circumference around this mark was recorded. Next, the children were instructed to let their arm hang by their side, and a caliper was used to measure their triceps skinfold thickness. After pulling a vertical skinfold, with the fingers 1 cm above the mark made earlier, the caliper was centred around the mark and placed to measure the skinfold. This measure was made in triplicate with a fourth measure made if prior measures differed by more than 2 mm. Together, these two measures were used to calculate the percent body fat as described by Ozturk et al. [221] (Equations 2.1-2.4). Though not perfect measures of adiposity, skinfold thickness, waist circumference, and BMI have been shown to have high correlation with gold standard methods of measuring adiposity in children [222]. Given the accessibility of the equipment needed for these measures, they were the most feasible options for the GDM study in this thesis.

Equations 2.1-2.4

1. $AMA\ (cm^2) = \frac{(MUAC - \pi TSF)^2}{4\pi}$
2. $AA\ (cm^2) = \frac{\pi}{4} \times \left(\frac{MUAC}{\pi}\right)^2$
3. $AFA\ (cm^2) = AA - AMA$
4. $\%\ Body\ Fat = \frac{AFA}{AA} \times 100$

Legend: AA: arm area; AFA: arm fat area; AMA: arm muscle area; MUAC: mid-upper arm circumference; TSF: triceps skinfold thickness

2.6. CV Outcomes

At the beginning of each assessment, the child's blood pressure was taken in triplicate. In addition, resting heart rates were recorded during the visit. Various other cardiac and vascular measures and indicators of health were employed in this project. Echocardiographic assessment of the child's heart and blood vessels was performed, imaging the heart in various planes and using different modalities, including 2D, M-mode, and Doppler imaging, the latter to assess blood flow and tissue dynamics, to provide a comprehensive picture of heart structure and function. The children were also situated with electrocardiogram (EKG) probes to measure the electrical activity of their hearts during the assessments.

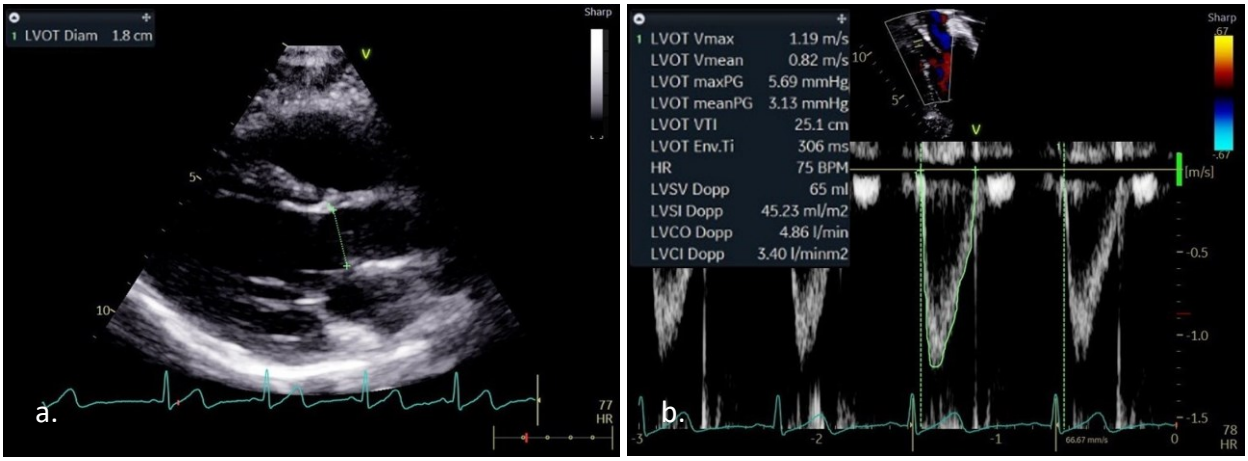
In addition to echocardiographic assessment, the EndoPAT 2000 system from Itamar Medical was used to investigate endothelial function. Together with vascular ultrasounds, these measures provide data regarding the children's vascular health, including arterial stiffness and endothelial function.

2.6.1. Cardiac Measures

Echocardiographic structural and functional assessment of the children's hearts included left ventricular (LV) measures of systolic and diastolic function as well as measures of the heart muscle thickness. Studies completed in Calgary were performed on General Electric (GE) Vivid S70 ultrasound machines while studies in Edmonton were completed using GE Vivid E95 machine, with vascular measures completed with a GE Vivid IQ system. The cardiac measures were completed by a senior echocardiography fellow in pediatric cardiology, while the vascular measures were completed by an experienced pediatric cardiologist. The measures taken included assessment of the LV outflow tract (LVOT), including dimensions and flow parameters, mitral valve (MV) hemodynamics using pulse wave Doppler, MV annular tissue Doppler assessment, LV dimensions, including the septal wall, posterior wall, interior diameter, and LV mass calculations, and LV shortening fraction [223].

The LVOT diameter measurement was made in the long axis view. The measure was made just below the aortic valve annulus shortly after closure of the MV, at its maximum diameter (Figure 2.1a). This measure was combined with an LVOT spectral Doppler trace from the 5-chamber view (Figure 2.1b) to calculate the stroke volume and cardiac output. Doppler trace measures were performed in triplicate to account for variability. Together, these measures give an indication of systolic function. As cardiac output and stroke volume change with age and body size, adjustment was also made by indexing to the children's calculated body surface area as recommended by the American Society of Echocardiography Pediatric and Congenital Heart Disease Council [223].

Figure 2.1. LVOT Echocardiographic Images

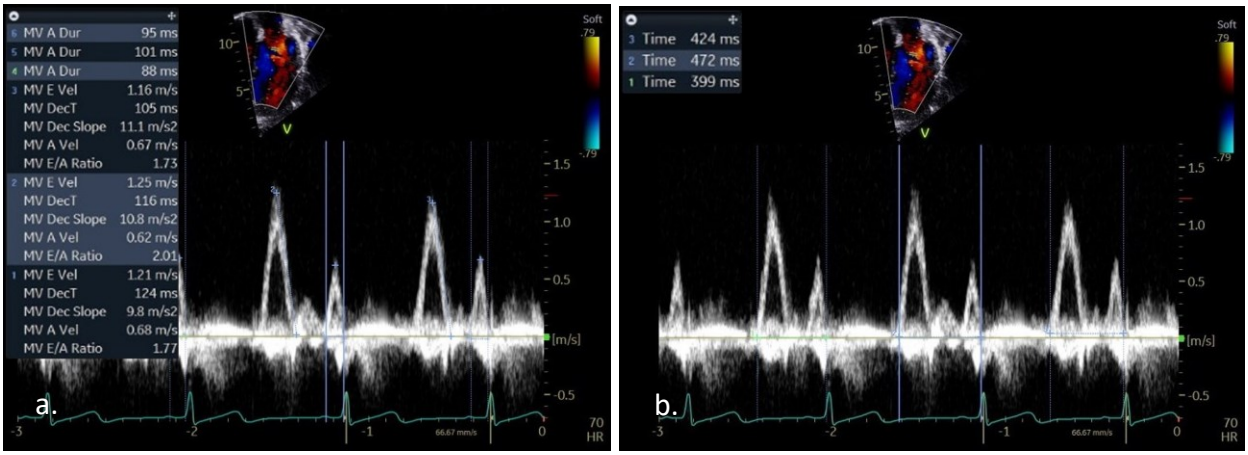


Legend: a. LVOT diameter from B-mode image; b. LVOT spectral Doppler trace; Env.Ti: time duration of the VTI trace; HR: heart rate; LVCI Dopp: cardiac output indexed to body surface area; LVCO Dopp: cardiac output; LVOT: left ventricular outflow tract; LVSI Dopp: LV stroke volume indexed to body surface area; LVSV Dopp: LV stroke volume; maxPG: max pressure gradient; meanPG: mean pressure gradient; Vmax: maximum flow velocity; Vmean: mean flow velocity; VTI: velocity time integral

MV spectral and tissue Doppler measurements give an indication of diastolic function. All MV Doppler measures were performed in triplicate. From MV spectral Doppler (Figure 2.2), we measured the E wave max velocity and deceleration time. The E wave measures passive blood flow into the LV during early diastole, with pressure gradient being driven by negative pressure in the LV when springing back to its resting state after systole. The A wave peak velocity was also measured, indicating the velocity of flow into the LV during atrial contraction. The E and A wave measures together were used to calculate the E/A ratio. The A wave duration was also measured. In mild forms of diastolic dysfunction, the E wave max velocity is reduced while the A wave velocity increases, the E/A ratio decreases, and deceleration time increases. More severe forms of diastolic dysfunction associated with heart failure would be unexpected in

these children. These measures are often limited by fast heart rates in children in which E and A waves can be fused [223], resulting in an augmented A wave and decreased E/A ratio [224]. Furthermore, these measures can vary with alignment of the transducer, sample placement, and the loading conditions of each heart [223].

Figure 2.2. MV Echocardiographic Spectral Doppler Images

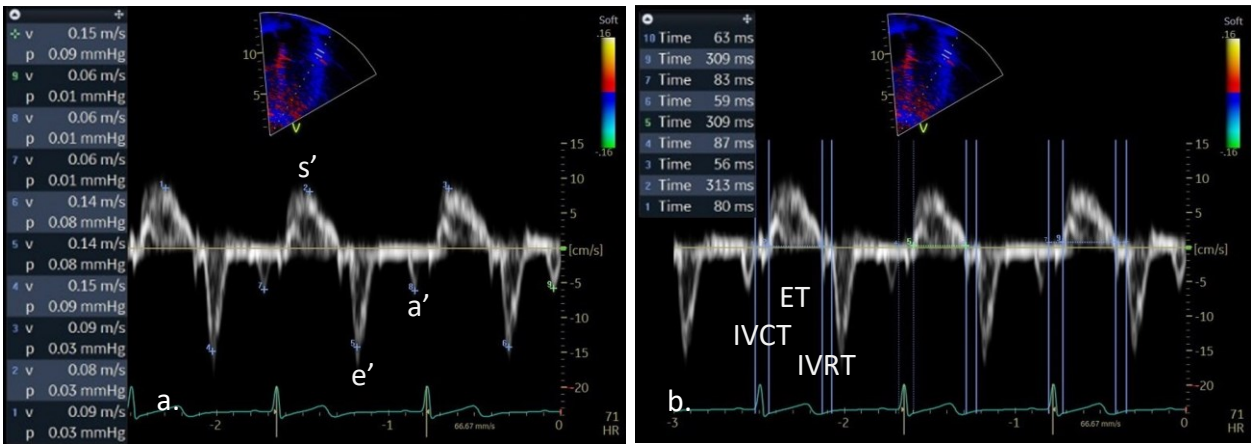


Legend: a. MV E wave, deceleration time, A wave, and A duration measures; b. MV inflow duration measures; A Dur: A wave duration; A Vel: A wave peak flow velocity; A wave: atrial contraction; DecT: E wave deceleration time; E Vel: E wave peak flow velocity; E wave: passive early filling; MV: mitral valve

MV tissue Doppler measures included measurement of the peak velocities of the medial and lateral walls of the LV, adjacent to the MV, during both systole and diastole. The movement of the tissue can be used to give additional information regarding systolic function. The s' wave was measured, indicating the peak velocity during systole, while the e' and a' waves indicated the peak tissue velocities during their respective portions of diastole (Figure 2.3a). The e' and a' wave max velocities follow similar patterns to the E and A waves in mild forms of diastolic dysfunction. MV tissue Doppler measures were also used to calculate the Tei index [225], which is

widely used as a measure of combined systolic and diastolic function. This is calculated by dividing the sum of the isovolumic contraction time (IVCT) and isovolumic relaxation time (IVRT) by the ejection time (ET). IVCT measures the amount of time from the end of the a' wave, marking the end of diastole, to the beginning of the s' wave, marking the beginning of systole. IVRT is measured from the end of the s' wave to the beginning of the e' wave, marking the end of systole to the beginning of diastole (Figure 2.3b).

Figure 2.3. MV Annulus Lateral Wall Echocardiographic Tissue Doppler Images

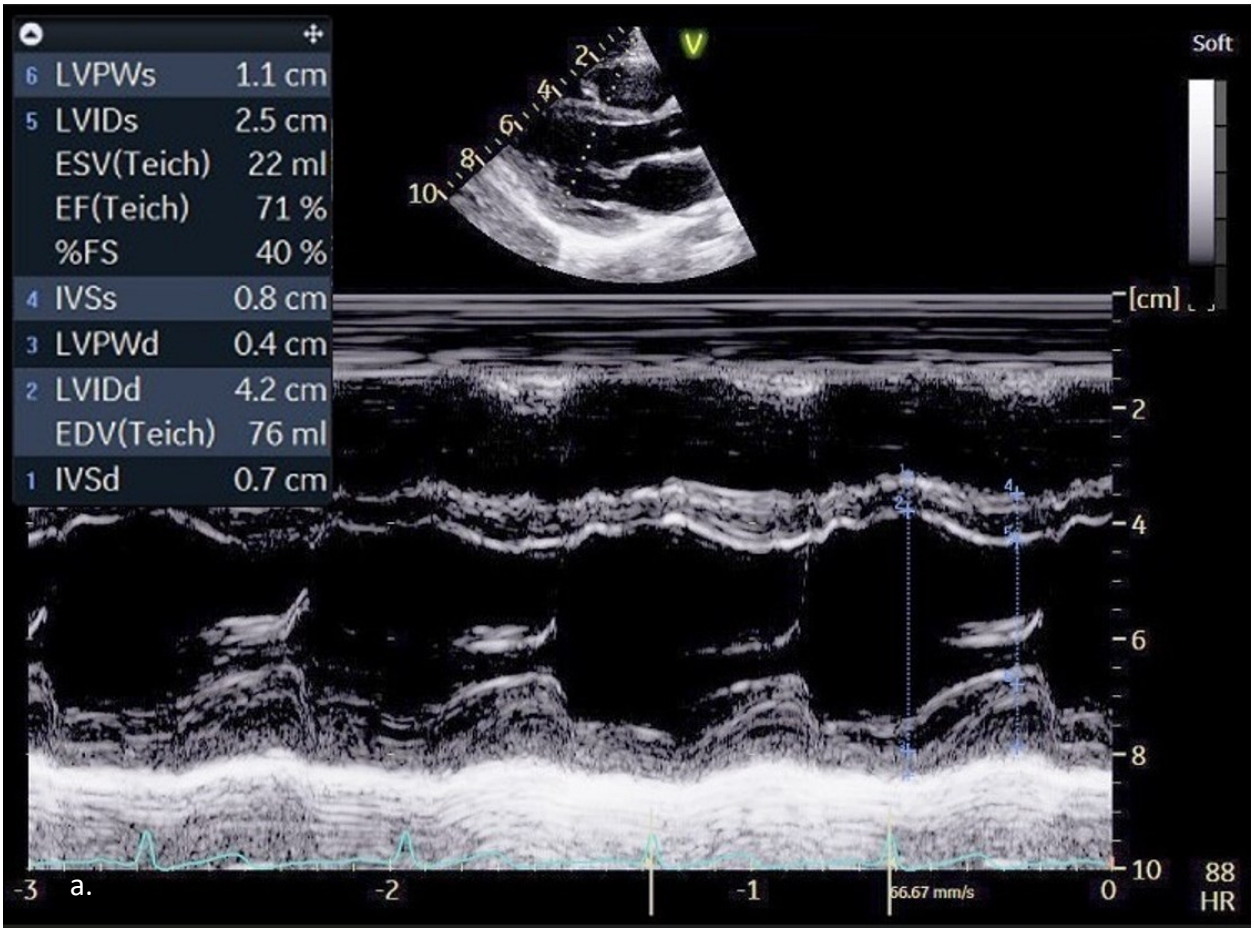


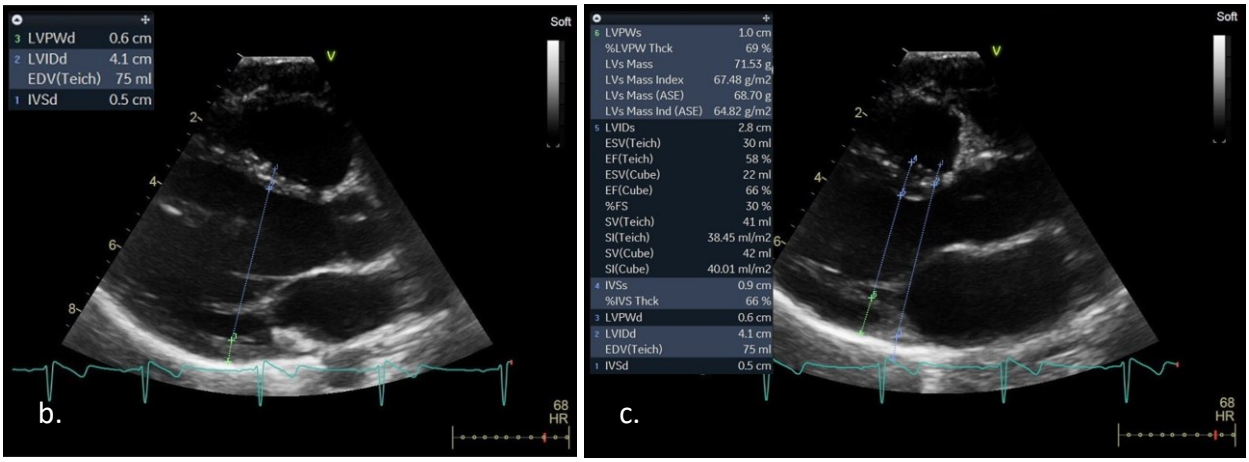
Legend: a. s', e', and a' waves; b. Tei index measures; a': MV annular peak tissue velocity during atrial contraction; e': MV annular peak tissue velocity during passive early filling; ET: ejection time; IVCT: isovolumic contraction time; IVRT: isovolumic relaxation time; MV: mitral valve; s': MV annular peak tissue velocity during systole

LV septal wall, posterior wall, and internal diameter dimensions were taken from M-mode images at end systole and end diastole (Figure 2.4a). These measurements were verified against B-mode images (Figure 2.4b & 2.4c), with B-mode measures taken in the place of M-mode measures when M-mode images lacked sufficient clarity or were otherwise invalid, and used to calculate shortening fraction—a measure of systolic function—and LV mass. Z scores

were generated for LV mass (for lean body mass [226]) and for interventricular septal and posterior wall thickness (for body surface area [227]). Using z scores rather than simply adjusting for lean body mass or body surface area provides a superior method of accounting for the effects of body size and age on cardiac dimensions [223], as the size of the heart increases with age and growth, but not at a constant rate. As suggested in the previous chapter, LV mass is a measure of particular interest, as LV hypertrophy is present in the fetuses of diabetic mothers and has been shown to persist, for some, into early childhood [122].

Figure 2.4. LV Dimensions from Echocardiographic Long-Axis Images





Legend: a. Parasternal long axis M-mode image; b. Parasternal long axis end-diastolic measures; c. Parasternal long axis end-systolic measures overlaid with end-diastolic measures; EDV: end-diastolic volume; EF: ejection fraction; ESV: end-systolic volume; FS: fractional shortening; IVSd: end-diastolic interventricular septal thickness; IVSs: end-systolic interventricular septal thickness; LV: left ventricle; LVIDd; end-diastolic internal LV diameter; LVIDs; end-systolic internal LV diameter; LVPWd: end-diastolic posterior LV wall thickness; LVPWs: end-systolic posterior LV wall thickness; SI: stroke volume indexed to body surface area; SV: stroke volume

Though not yet analyzed in the present study due to technical difficulties during image transfer, myocardial strain has been established as a measure of LV function and has been shown to be effective in detecting subclinical dysfunction when other parameters give inconsistent results [228–232]. Longitudinal strain measures changes in LV dimensions from the base of the heart to the apex throughout the cardiac cycle. Circumferential strain measures strain around the LV in the short axis view, and radial strain measures changes in the thickness of the heart muscle. As reviewed by Koopman et al. [231], strain assessment by speckle tracking helps overcome important limitations of other echocardiographic measures. As opposed to spectral or tissue Doppler imaging and M-mode imaging, strain measurements are able to track motion in all directions and are independent of the imaging plane. Strain rate has an additional advantage over other

measures of cardiac function, as it is less affected by loading conditions [233]. Strain analyses would contribute additional understanding to the main study of this thesis.

2.6.2. Vascular Measures

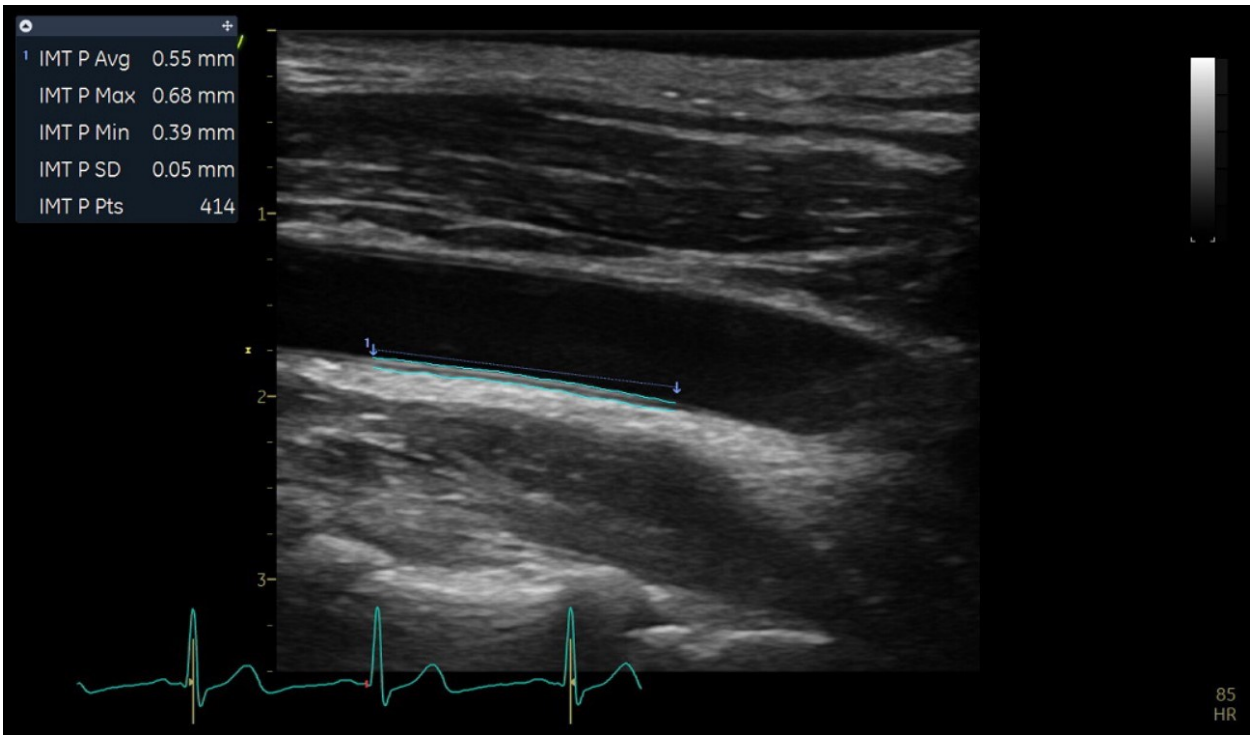
Vascular health begins in childhood and tracks into adulthood. Commonly used measures of vascular health in children include carotid intima-media thickness (IMT), measures of endothelial function, and arterial compliance (using distensibility or pulse wave velocity measures) [234]. In the main study of this thesis, the study team elected to use multiple measures of vascular health, in both central and peripheral arteries, to obtain a clear picture of overall vascular health. These measures, including alternative methods, strengths, and weaknesses, are described in detail below.

2.6.2.1. Carotid IMT and Distensibility

Carotid IMT (cIMT) has been established as a measure of the degree of vascular remodeling and atherosclerosis [235]. Measurement of cIMT can be limited by the number of points measured, as IMT can be variable depending on the degree of remodeling in different areas of the carotid artery. As such, our team elected to use a linear ultrasound probe and GE software to automate cIMT measurements. cIMT was measured on the posterior wall of the left common carotid artery. Using the software, a length of the posterior wall was selected, at a time point between the P wave and QRS complex of the EKG tracing, and the boundaries of the intima-medial area were detected within the selection (Figure 2.5). Measurements were made on the posterior wall to minimize the interference of surface pressure from the transducer on the measure. A trained research assistant performed all cIMT measures and visually confirmed that

the boundaries were correctly detected. The number of points included in the cIMT averages for each participant ranged from 225 to 564, assuring much greater accuracy than if just a few points were used.

Figure 2.5. Carotid IMT Measurement on Echocardiographic Imaging with a Linear Probe



Legend: IMT: intima-media thickness; P Avg: average posterior wall thickness; P Max: maximum posterior wall thickness; P Min: minimum posterior wall thickness; P Pts: number of individual posterior wall thickness measures; P SD: standard deviation of posterior wall thickness measures

In addition to cIMT, the systolic (maximum) and diastolic (minimum) diameters of the common carotid artery were measured in B mode images and used to calculate carotid compliance, pressure-strain elasticity modulus, as well as the carotid β-stiffness index, as described previously [236,237]. The equations used are given below (Equations 2.5-2.7), with lengths

in mm and pressures in kPa, measured using brachial cuffs. The β-stiffness index has long been used as an indicator of arterial stiffness and cardiovascular disease [238] and has been used in children [239–241].

Equations 2.5-2.7

5. $$Elast_{P-S} = \frac{D_d \times \Delta P}{\Delta D}$$

6. $$Compliance = \frac{\pi\left(D_s^2 - D_d^2\right)}{4\Delta P}$$

7. $$\beta = \frac{\ln\left(\frac{P_S}{P_d}\right)}{\left(\frac{\Delta D}{D_d}\right)}$$

Legend: β: carotid beta stiffness index; ΔD: change in the common carotid artery diameter from systole to diastole; ΔP: brachial cuff pulse pressure; D_d: diastolic (minimum) diameter of the common carotid artery; D_s: systolic (maximum) diameter of the common carotid artery; $Elast_{P\text{-}S}$: pressure-strain elasticity modulus; P_d: brachial cuff diastolic blood pressure; P_s: brachial cuff systolic blood pressure

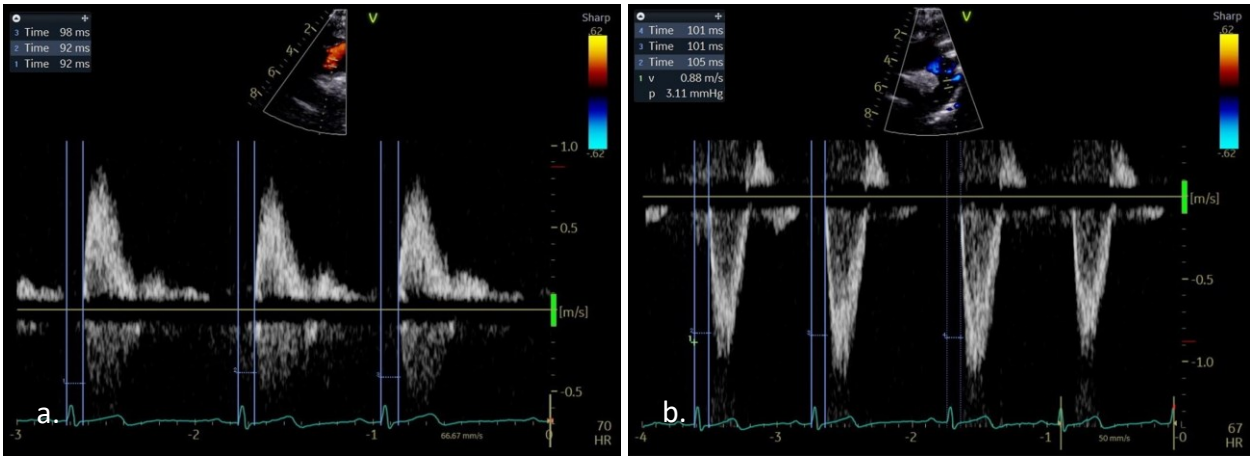
2.6.2.2. Aortic and Peripheral Pulse Wave Velocity

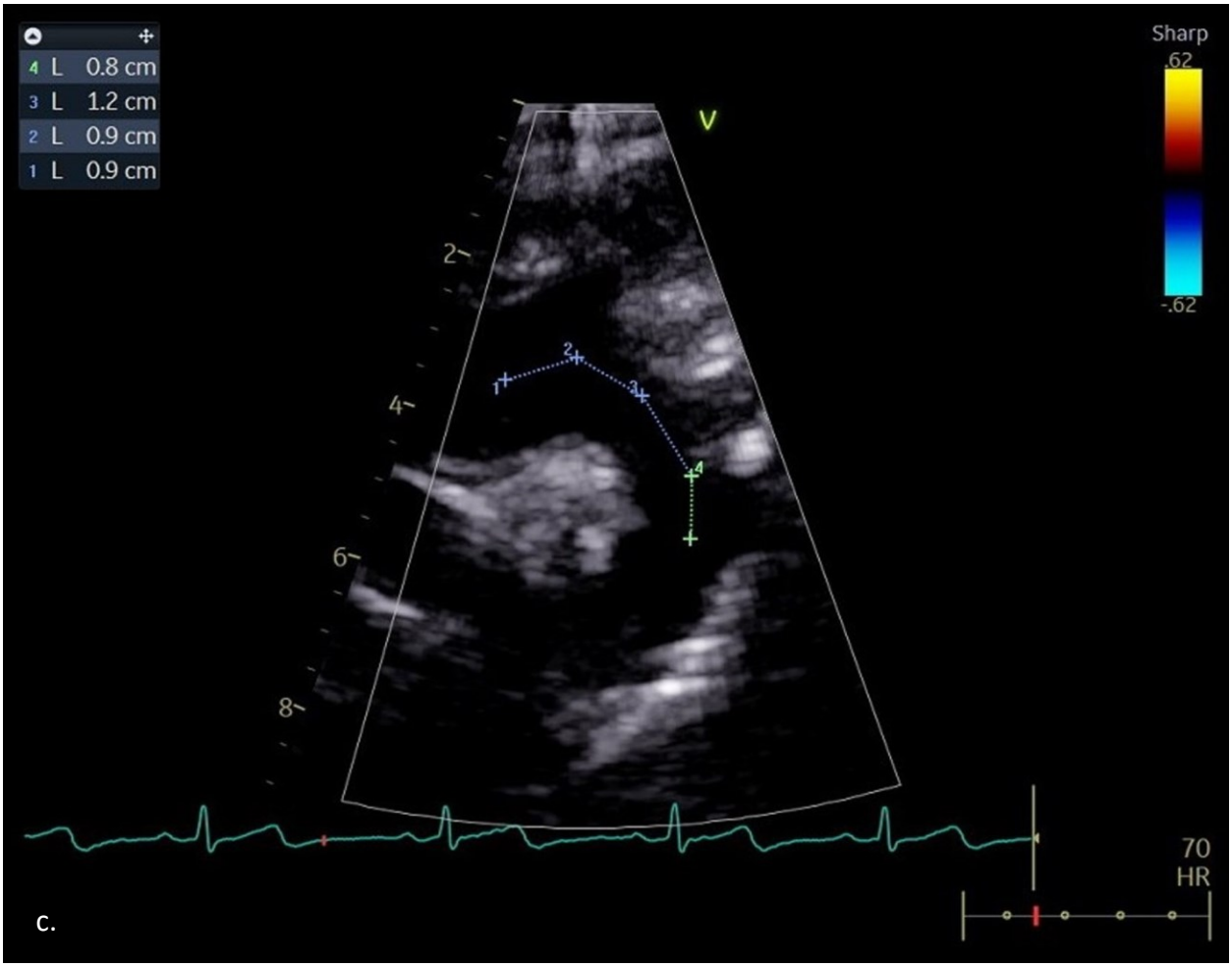
In addition to the carotid β-stiffness index, this study included echocardiographic measures of aortic and peripheral pulse wave velocity (PWV). PWV measures how quickly pressure waves are transmitted through the arteries, with a faster velocity indicating greater stiffness of the arteries. Probe-based carotid to femoral PWV is currently the gold standard of measuring arterial stiffness, though magnetic resonance or Doppler flow can also be used to detect flow waveforms as described by Wilkinson et al. [242]. Though magnetic resonance imaging gives a more accurate measure of the path length, the temporal resolution is lower, and it is very expensive. For the main study of this thesis, the study team opted to use ultrasound-based

measurement of PWV, primarily due to the ease of access to ultrasound equipment, as echocardiography equipment was used both for assessing the heart and completing vascular measures. Two different PWV measures were taken in this study: aortic (proximal to distal aortic arch) and peripheral (carotid to posterior tibial arteries).

To calculate aortic PWV, spectral Dopplers were obtained from both the proximal and distal aortic arch. Simultaneous EKG recordings were made. At each point of the aorta, the time from the onset of QRS to the onset of flow was recorded, averaged over three consecutive cycles (Figure 2.6a & 2.6b). The difference between these times was calculated to indicate the transit time. The distance from the proximal to the distal points measured by the spectral Dopplers was approximated (Figure 2.6c), and the aortic PWV was calculated as the distance divided by the transit time.

Figure 2.6. Aortic PWV Measurement on Echocardiographic Imaging

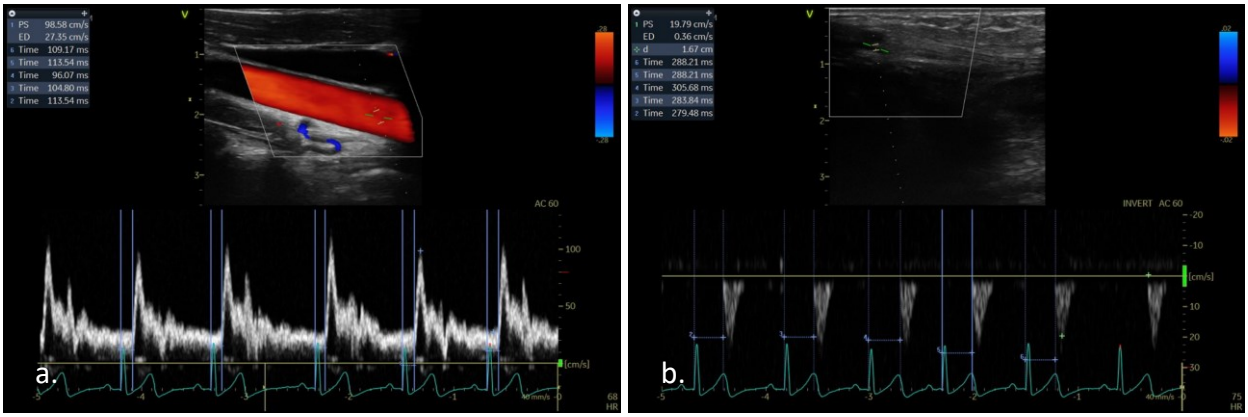




Legend: a. Proximal time to flow; b. Distal time to flow; c. Proximal to distal length; PWV: pulse wave velocity

Peripheral PWV measurements were calculated in the same fashion but with the Doppler readings taken from the common carotid artery and the posterior tibial artery (Figure 2.7), and the distance between the two points was approximated with an external tape measure.

Figure 2.7. Peripheral PWV Measurement on Echocardiographic Imaging



Legend: a. Common carotid time to flow; b. Posterior tibial time to flow; PWV: pulse wave velocity

Additional factors that may influence pulse wave velocity include heart rate, blood pressure, and fasting state [243–245]. Differences between groups in average heart rate and blood pressure will need to be considered when comparing means in the main study of this thesis. As for fasting state, participants were instructed to arrive for appointments having fasted for at least 3 hours, with the majority of participants adhering to this guidance. This again provides consistency for comparisons between the groups. Additionally, that PWV measures in this study were taken serially rather than instantaneously may have had an influence on the results. However, the measurements were taken very close together temporally, so this is unlikely to have had a large influence.

2.6.2.3. EndoPAT Test of Endothelial Function

The endothelium, the inner lining of blood vessels, plays a crucial role in vascular function and health. The endothelium acts as a barrier between circulating blood and the vessel wall and regulates the exchange of various elements. In addition to this crucial role, the endothelium also plays an important role in vasoconstriction and dilation, essential for regulating

blood flow [246]. Different tests have been used to assess endothelial function, but results have shown that reduced endothelial function is correlated with risk of CV events and mortality [247].

The gold standard of measuring endothelial function involves intracoronary infusion of vasoactive agents, as described by Tousoulis et al. [248]. Given the invasiveness of this procedure, other alternatives are commonly used. The flow mediated dilatation method uses ultrasound images to measure changes in the diameter of the brachial artery following occlusion and is the most commonly used non-invasive test. This test requires careful standardization, however, and can have high variability between measures and observers [248–250].

The EndoPAT test, which was the test chosen for the main study of this thesis, represents another alternative that is automated, less operator dependent, and has been shown to have high test-retest reliability [249]. Digital peripheral arterial tonometry (PAT) assesses changes in arterial pulse amplitude using finger plethysmography sensors before and after occluding blood flow. This gives an indication of endothelial and microvascular function [251]. The EndoPAT test measures the reactive hyperemia index (RHI), the ratio of the pulse amplitude after occlusion to the pulse amplitude before occlusion, with higher RHI scores indicating better endothelial function. EndoPAT test results correlated with coronary endothelial dysfunction [252] and have been shown to predict risk of CV events [253]. The test has been shown to be tolerated well by children [254]. Furthermore, differences in EndoPAT test results have been shown in children with vascular pathologies versus controls [255], and poor EndoPAT test results have been shown to correlate with poor glycemic controls in children with diabetes [256]. Thus, the EndoPAT test is a feasible and valid test of endothelial function in children for our main study.

Children were instructed to fast for 4 hours prior to the start of their EndoPAT tests, only drinking water. The majority (45/52) followed this guideline, with no participants fasting for less

than 2 hours. Due to occlusion of the brachial artery when taking blood pressure measurements, blood pressure was taken at the start of each study visit, before the child's echocardiogram, and the EndoPAT test was completed at the end. This allowed sufficient time for any reactive hyperemic response due to blood pressure measurements to subside before beginning the EndoPAT test. Tests were performed in a quiet, dimly lit room, at a comfortable temperature. Children were instructed to lay on their backs and stay as still and relaxed as possible in order for clear readings to be obtained. A blood pressure cuff was placed on the right arm, probes were placed on both pointer fingers, and the hands were positioned so that the fingers were not in contact with the child's body or other fingers. The tests were run in standby mode for one minute prior to commencing to ensure that a clear reading was being obtained, and adjustments were made as necessary. After the tests began, the children lay still for five minutes for a baseline measurement. Their right brachial artery was then occluded for 5 minutes at 60 mm Hg above their systolic blood pressure. After this period of occlusion, the pressure was released, and the children lay still for a final 5-minute period. RHI scores were obtained using the EndoPAT software, with adjustments made to automatic occlusion borders as necessary. The borders were only manually changed if they were off by 9 or more cycles at the beginning of occlusion or by 4 or more cycles at the end of occlusion. The RHI scores were normalized to obtain lnRHI scores.

Chapter 3: The Impact of Gestational Diabetes on the Cardiovascular Health of the Child

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3.1. Introduction

Cardiovascular disease (CVD) represents the most common cause of death globally, accounting for nearly one-third of deaths worldwide [1]. The majority of these deaths are due to myocardial infarction and cerebrovascular events, acute events frequently caused by underlying atherosclerotic CVD, but other manifestations of CVD also present a significant burden in terms of morbidity and costs [16–19]. Risk factors that are understood to contribute to the progression of CVD include high cholesterol, hypertension, smoking, and diabetes, factors applied to the generation of Framingham Risk Scores for CVD [22].

Over the past two decades there has been growing animal [257,258], clinical [51,69,122,133,259–261], and population-based [27,29,262] evidence that adverse exposures early in life, such as restricted fetal growth or preterm birth, result in a change in the homeostasis of certain organ systems, in part to support fetal growth and health, but at the expense of contributing to longer-term disease. With respect to long-term CVD, this is known as fetal cardiovascular (CV) programming [263]. Diabetes in pregnancy represents another adverse prenatal exposure which predisposes infants to both shorter- and longer-term disease risks. In utero exposure to maternal diabetes, both pregestational and gestational (GDM), has been shown to increase cardiometabolic disease risks in the offspring [108,109,111,264]. However, the mechanisms, contributing pre- and postnatal factors, and the timing of development of CVD are not completely understood.

Maternal diabetes has been associated with both cardiac and vascular changes through fetal stages, in infancy, and into childhood, which may be related to later development of CVD. Fetal ventricular hypertrophy and altered diastolic function are common features witnessed in diabetic pregnancies [114–117], with left ventricular (LV) hypertrophy persisting through infancy [117,133] and into childhood and adolescence [122,261]. Vascular changes reported have include increased aortic compliance in utero [265], increased aortic intima-media thickness (IMT) in newborns [132], and increased aortic stiffness in children [122].

While these outcomes have been explored in children whose mothers had diabetes prior to pregnancy, little work has been done to understand the evolution of CVD in children (CGDM) of mothers with GDM. As GDM affects a large proportion of pregnancies [91] and is on the rise in Canada [113], it is important to understand its impact. In fact, findings from Do et al. [122,133] in children whose mothers had pregestational diabetes indicated a positive correlation between third trimester hemoglobin A_{1c} with aortic stiffness and LV mass of the child. This suggests that exposure in the latter half of gestation to the intrauterine milieu of the diabetic pregnancy may be sufficient in pregnancies complicated by GDM to result in altered CV health in the offspring even early in life.

In addition to GDM exposure, other pre and postnatal factors may also contribute to the child's CV health. Such factors include maternal diet and metabolic status in pregnancy and the child's own diet and physical activity. Nutrient intake in pregnancy has been examined, and specifically, studies have found higher maternal carbohydrate intake in pregnancy to be associated with higher blood pressure in the offspring [149,150] and maternal protein intake and carotid IMT (cIMT) to be negatively correlated [266,267]. Gale et al. [267] found that the negative association between maternal protein intake and the child's cIMT may be explained by mother's

reduced overall energy intake; however, the negative association of protein intake with cIMT persisted in another study that adjusted for overall energy intake [266]. In addition, both human and animal studies have highlighted the importance of the child's diet [160,162,163] and exercise habits [164–167] to lower CVD risk and modulate the impact of negative early exposures.

Elevated iron—an important nutrient in fetal growth and development, yet an agent in generating oxidative stress—has been observed in women with GDM [151–156], hypothesized to induce beta cell dysfunction and apoptosis, decreased insulin secretion, and lead to greater insulin resistance [268–270]. Elevated iron status has been implicated in the progression of atherosclerotic CVD in adults, through lipid peroxidation or other mechanisms [271]. Studies in rat models have consistently found that low maternal iron in pregnancy leads to elevated systolic blood pressure in the adult offspring [272–274], exacerbated by poor postnatal diet in the offspring [275]. Clearly iron intake and status is an important factor in both the mother and child's health, with implications for the child's long-term CV health, but associations between maternal iron and CV health in the child have not yet been fully explored.

In the present study, we 1) explore the CV health of CGDM with comparison to age and sex matched children of healthy mothers, 2) explore the relationship between maternal diet and iron status in GDM pregnancies and the CV health of the child, and 3) investigate associations between the child's dietary intake, metabolic status, and exercise and their CV health. We hypothesize that CGDM have increased arterial stiffness and LV mass associated with a need for maternal insulin therapy for GDM management. We further hypothesize that poor maternal diet quality and high iron status as well as poor metabolic health and less physical activity of the child will be associated with worse CV health of the child, particularly among CGDM.

3.2. Methods

This study is a nested, matched prospective case-control study, tied to an established birth cohort study in the province of Alberta, with participants located primarily in the two major cities (Calgary and Edmonton). Ethical approval was received from both the Health Research Ethics Board - Health Panel at the University of Alberta (Edmonton) and the Conjoint Health Research Ethics Board at the University of Calgary prior to beginning the study. Participants were recruited between September 2021 and March 2022. Healthy CGDM were identified through the Alberta Pregnancy Outcomes and Nutrition (APrON) Cohort Study [168,180,181,276]. Women had been recruited into the APrON study from May 2009 to June 2012 in pregnancy and followed through the birth and subsequent years [182], with the children of these pregnancies ranging currently in age from 9 to 12 years. CGDM were excluded if they were born prior to 36 weeks or had a birth weight <5th percentile. Children born to healthy mothers without GDM and following an uncomplicated pregnancy served as controls. Controls were matched for the child's year of age and biological sex as well as maternal age, pre-pregnancy body mass index (BMI), weight gain during pregnancy, and parity at the time of enrollment into the original APrON study. After informed consent was received, data regarding each mother's and child's health histories, demographics, and nutrition were collected, both retrospectively through the APrON study and prospectively, with additional prospective data regarding the child's physical activity, and a single CV assessment was completed either in Calgary or Edmonton depending on the participant's location of residence.

3.2.1. GDM Status – Mothers

Maternal GDM status was self-reported through APrON study questionnaires, and this data was used for initial identification of cases. This was verified during the initial conversation with participants as part of a screening questionnaire. Medical records were reviewed to confirm GDM screening test results and record use of insulin during the pregnancies. All screening and diagnostic tests used cut-off glucose values recommended in 2008 clinical practice guidelines [188]. For this study, women with either a diagnosis of impaired glucose tolerance or GDM were considered as having had GDM, in closer alignment with current 2018 guidelines [187].

3.2.2. APrON Study Data – Mothers and Children

Detailed data regarding the mothers were provided by the APrON study, including maternal education and level of income, past medical history, metabolic status in pregnancy, including biomarkers of iron status and diabetes screening test results, and dietary intake during pregnancy. Data were collected by the APrON study in each trimester of pregnancy and at specified time points postnatally as described by the APrON study team [276]. Maternal dietary intake had already been assigned scores to reflect adherence to the Canada Food Guide (CFG) and intake of less healthy foods [195]. In addition to these data, the APrON study also provided details regarding the child's birth history including gestational age at birth, birth weight, and admission to the neonatal intensive care unit immediately after birth.

3.2.3. Blood Pressures & Biometric Data – Children

For each child, right arm blood pressure (measured in triplicate; mean values reported), height, weight, waist, mid upper arm circumference, and triceps skinfold thickness (in triplicate;

mean value reported) were all measured by a trained study team member (CB). The child's height and weight were used to calculate BMI z scores based on their age and biological sex using an online calculator based on the Centers for Disease Control and Prevention reference charts [217]. Lean body mass was calculated using validated, sex-specific equations, using the child's height, weight, and BMI z score [218]. Waist circumference was used to calculate waist-to-height ratio. Percent body fat was calculated using mid upper arm circumference and triceps skinfold thickness measures as described by Ozturk et al. [221].

3.2.4. Dietary Intake Data – Children

Following the visits, and with parental support, the children were instructed to complete three days of 24-hour food recalls. Logbooks were also used to facilitate participants' ability to recall food and beverages consumed, but these were not collected by the study team. Dietary intake data for 24-hour recalls were collected and analyzed using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool version 2018-Canada (National Cancer Institute, Bethesda, MD, USA). The ASA24 system has been validated extensively [204,277–279]. Data were analyzed for total daily energy, macronutrient, and micronutrient intakes. Nutrition data was further analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to generate child-specific Healthy Eating Index (HEI) scores [206,208].

3.2.5. Physical Activity – Children

The children were asked to wear ActiGraph (Pensacola, FL, USA) wGT3X series accelerometers on the right side of their hips using elastic waistbands for 7 days following the visits. They were instructed to remove the devices when sleeping or participating in water

activities. Children completed activity logs to document when the device was worn during the day and activities completed when the devices were removed. Physical activity data were analyzed using ActiLife version 6.11.9 software (ActiGraph). The devices were initialized with a sampling frequency of 30 Hz and set to record data in 15 second epochs. Evenson's cut-off points [210] were used to classify the child's physical activity levels (sedentary, light, moderate, or vigorous) as recommended in a comparative study in children [211]. Wear time was validated using 10 hours per day and 5 days as the minimum and defining non-wear period as 45 minutes or more. After data validation, average time per day spent in moderate to vigorous physical activity (MVPA) was calculated.

3.2.6. Echocardiography – Children

During the in-person visits, complete anatomical and functional echocardiographic assessments were performed [223], using General Electric (GE) Vivid S70 and E95 systems (General Electric, Boston, MA, USA), with offline analysis completed using GE EchoPAC software. Measures included LV posterior wall (LVPWd) and interventricular septal (IVSd) diastolic wall thickness, shortening fraction, spectral Doppler E and A wave mitral inflow velocities (averages of three cycles), tissue Doppler mitral valve annular velocities (s', e', and a'; averages of septal and lateral wall measures over three cycles), E/A wave ratios, isovolumic relaxation (IVRT) and contraction (IVCT) times, and ejection times (ET). IVRT, IVCT, and ET were all measured on tissue Doppler images, and the Tei index was calculated as a measure of global systolic and diastolic function, as previously described [225]. LV mass calculations were made using the Devereaux method and normalized for lean body mass, generating z scores [226]. In

addition, z scores for IVSd and LVPWd measures were generated, normalized by body surface area [227].

3.2.7. Carotid IMT and Vascular Stiffness – Children

Vascular measures were completed using a GE Vivid IQ system (General Electric, Boston, MA, USA) with a 12 MHz linear transducer. Two-dimensional images were taken of the left common artery, and an automated vascular program was used to measure the intima-medial thickness of the posterior wall. In addition, the carotid diameter in systole and diastole was measured and used to calculate the pressure-strain elasticity modulus, arterial compliance, and β-stiffness index as previously described. [237].

Aortic (aPWV) and peripheral (pPWV) arterial pulse wave velocity (PWV) were assessed as measures of arterial stiffness using an echocardiographic Doppler method [122,280,281]. For aPWV measures, proximal and distal spectral Doppler tracings were obtained along the aortic arch from a suprasternal notch view sequentially, and the aortic arch length was measured between these two points using serial measurements. Using simultaneous electrocardiographic tracings, times from onset of the QRS waveform to onset of flow at each point was measured and averaged over three cycles. Time to flow in the proximal site (T1, either the ascending aorta for aPWV or the left common carotid artery for pPWV) was subtracted from time to flow in the distal site (T2, distal arch for aPWV or the posterior tibial artery for pPWV) to calculate the transit time (T2-T1). aPWV was calculated by dividing the distance between sample sites along the aortic arch by the transit time: Distance/(T2-T1) (m/s). pPWV measurements and calculations were made following a similar procedure, but with spectral Doppler tracings placed

sequentially on the carotid and posterior tibial arteries and the direct length measured externally using a tape measure between the 2 sites.

3.2.8. Peripheral Artery Endothelial Function – Children

Endothelial function was measured using the EndoPAT 2000 system (Itamar Medical, Caesarea, Israel) [254–256,282]. Children were instructed to arrive for EndoPAT tests having fasted for four hours. Baseline blood pressure measurements were taken at least 30 minutes prior to beginning endothelial function assessments in order to avoid any interference with test results. Tests were performed in quiet, dimly lit rooms with children in the supine position, and they were instructed to stay as still and relaxed as possible throughout the tests. Children were asked to remove any jewelry or articles of clothing that could possibly restrict blood flow, and peripheral arterial tonometry probes were placed on both index fingers. A blood pressure cuff was positioned around the right upper arm prior to beginning each test. Tests began with a five-minute baseline measurement followed by 5 minutes of occlusion and a subsequent 5-minute post-occlusion period. The blood pressure cuff was inflated to 60 mmHg above systolic blood pressure throughout the five-minute occlusion period. Once tests were completed, occlusion windows were detected automatically using EndoPAT software and adjusted as required. Adjustments were only made if the start of occlusion was off by 9 cycles or more or if the end of occlusion was off by 4 cycles or more. Reactive Hyperemia Index (RHI) scores were generated as the ratio of post-occlusion amplitude to baseline amplitude and normalized using the logarithmic function (lnRHI).

3.2.9. Primary and Secondary Outcomes

For the purposes of the analyses, primary and secondary outcomes were defined. Primary outcomes were LV mass z score, lnRHI, aPWV, pPWV, and cIMT. Secondary outcomes consisted of systolic and diastolic blood pressure and resting heart rates as well as IVSd and LVPWd measurements, systolic, diastolic, and global function, and vascular measures in the carotid artery.

3.2.10. Statistical Analysis

Participant baseline and demographic information were summarized using frequencies for categorical variables and mean (standard deviation), median (interquartile range), and range for numerical variables as appropriate. Possible differences in primary and secondary CV outcomes between groups were explored using chi-squared tests or Fisher's exact test (for small frequencies) for categorical and t-tests or Wilcoxon rank sum (for heavily skewed data) for numerical data. Independent t-tests were used to compare CV measures between the experimental and control groups. Additional sub-analysis was completed comparing diet versus medication for glycemic control. Further, differences between groups in measures of maternal dietary intake and iron status during pregnancy were investigated as well as differences in the children's nutrition and physical activity. Data were explored using univariate linear regression models to examine relationships between maternal and child exposures and primary CV outcomes. These models were then used to build multiple regression models, including relevant covariates as indicated in the univariate models, along with clinically significant variables. Regression analyses and ANOVA were completed using SAS 9.4 software (SAS Institute Inc.

Cary, NC, USA). All other analyses were completed using SPSS Statistics for Windows version 28 (IBM, Armonk, NY, USA).

3.3. Results

Twenty-five mothers who had GDM in their pregnancies and 27 mothers who did not (controls), along with their children, were included in the analyses. There was one set of twins in the group whose measures were averaged, and the mother/child dyad treated as one unit of analysis. Thirty-five children resided in the Calgary area (20 CGDM and 15 controls) and 17 in the Edmonton area (5 CGDM and 12 controls). Of the mothers with GDM, for 17, their GDM was managed through diet alone, whereas 8 required insulin therapy. Groups were comparable with respect to the mothers' (age, parity, pre-pregnancy BMI, and weight gain during pregnancy) and children's (biological sex and age) characteristics, with the child's age at the appointment being the only characteristic with a statistically significant difference (CGDM vs controls; 11.1±0.7 vs 10.7±0.6 years, $p=0.03$) (see Tables 3.1 & 3.2).

Table 3.1 displays additional maternal characteristics, including maternal education and current family income, comparing control mothers and mothers whose pregnancies were complicated by GDM, including all GDM cases, diet controlled, and insulin controlled. There were no statistically significant differences among groups in these measures. None of the mothers included in the study reported smoking in their pregnancies, one reported alcohol consumption early in the pregnancy, and none reported use of recreational drugs. Two control mothers reported having polycystic ovary syndrome.

In Tables 3.2 and 3.3, participant demographics, other baseline variables, and current anthropometrics are shown. Demographic and other baseline measures were statistically

indistinguishable between CGDM and control groups, aside from the difference in age previously noted (Table 3.2). Upon further breakdown of CGDM into diet versus insulin managed, the age difference between CGDM whose mother's GDM was managed through diet alone and controls was attenuated. Anthropometric measures of the children were similar among groups as well, with no statistically significant differences (Table 3.3).

Table 3.1. Maternal Variables for CGDM Versus Controls

Maternal Variables	Total (n=52)	Control (n=27)	GDM (n=25)	p[a] value	GDM Diet (n=17)	p[b] value	GDM Ins (n=8)	p[c] value	p[d] value
Age at Delivery (years)	34±4	34±4	35±5	0.66	34±5	0.96	36±4	0.37	0.47
Nulliparous	25 (48.1%)	15 (55.6%)	10 (40.0%)	0.26	8 (47.1%)	0.58	2 (25.0%)	0.23	0.40
Pre-pregnancy BMI Group				0.86		1.00		0.46	0.64
Normal weight	23 (44.2%)	12 (44.4%)	11 (44.0%)		7 (41.2%)		4 (50.0%)		
Overweight	16 (30.8%)	9 (33.3%)	7 (28.0%)		6 (35.3%)		1 (12.5%)		
Obese	13 (25.0%)	6 (22.2%)	7 (28.0%)		4 (23.5%)		3 (37.5%)		
Weight Gain in Pregnancy				1.00		1.00		1.00	1.00
Below	41 (87.2%)	23 (85.2%)	18 (90.0%)		12 (85.7%)		6 (100%)		
Within	6 (12.8%)	4 (14.8%)	2 (10.0%)		2 (14.3%)		0 (0%)		
Above	0 (0%)	0 (0%)	0 (0%)		0 (0%)		0 (0%)		
Maternal Education				1.00		0.79		0.40	0.11
High school	6 (11.8%)	3 (11.5%)	3 (12.0%)		1 (5.9%)		2 (25.0%)		
Trade or technical	11 (21.6%)	6 (23.1%)	5 (20.0%)		3 (17.6%)		2 (25.0%)		
University	22 (43.1%)	11 (42.3%)	11 (44.0%)		10 (58.8%)		1 (12.5%)		
Post-graduate	12 (23.5%)	6 (23.1%)	6 (24.0%)		3 (17.6%)		3 (37.5%)		
Current Family Income				0.055		0.19		0.26	1.00
20K-39,999	0 (0%)	0 (0%)	0 (0%)		0 (0%)		0 (0%)		
40K-69,999	5 (9.6%)	5 (18.5%)	0 (0%)		0 (0%)		0 (0%)		
70K-99,999	6 (11.5%)	4 (14.8%)	2 (8.0%)		2 (11.8%)		0 (0%)		
100K or more	41 (78.8%)	18 (66.7%)	23 (92.0%)		15 (88.2%)		8 (100%)		

Legend: BMI: body mass index; CGDM: children of mothers who had gestational diabetes; GDM: gestational diabetes; Ins: insulin. **P values:** a. GDM vs Control; b. GDM Diet vs Controls; c. GDM Insulin vs Controls; d. GDM Diet vs Insulin

Table 3.2. Birth Characteristics, Age, and Adverse Postnatal Life Exposures for CGDM Versus Controls

Child Variables	Total (n=52)	Control (n=27)	CGDM (n=25)	p[a]	CGDM Diet (n=17)	p[b]	CGDM Ins (n=8)	p[c]	p[d]
Birth/General									
Biological Sex - Female	40 (76.9%)	20 (74.1%)	20 (80.0%)	0.61	13 (76.5%)	1.00	7 (87.5%)	0.65	1.00
Birth Weight (kg)	3.28±0.49	3.32±0.49	3.23±0.51	0.54	3.24±0.55	0.62	3.22±0.42	0.62	0.95
Gestational Age (weeks)	39.0±1.3	39.2±1.4	38.8±1.2	0.23	38.9±1.3	0.49	38.5±0.8	0.16	0.40
NICU Admission	4 (7.7%)	3 (11.5%)	1 (4.0%)	0.61	1 (5.9%)	1.00	0 (0%)	1.00	1.00
At Time of Appointment									
Age (years)	10.9±0.7	10.7±0.6	11.1±0.7	0.03	11.0±0.7	0.16	11.4±0.7	0.01	0.21
Number of ALE				0.91		0.89		1.00	1.00
0	30 (57.7%)	16 (59.3%)	14 (56.0%)		9 (52.9%)		5 (62.5%)		
1	19 (36.5%)	9 (33.3%)	10 (40.0%)		7 (41.2%)		3 (37.5%)		
2	3 (5.8%)	2 (7.4%)	1 (4.0%)		1 (5.9%)		0 (0%)		

Legend: ALE: adverse life experiences; CGDM: children of mothers who had gestational diabetes; Ins: insulin; NICU: neonatal intensive care unit. **P values:** a. CGDM vs Control; b. CGDM Diet vs Controls; c. CGDM Insulin vs Controls; d. CGDM Diet vs Insulin

Table 3.3. Anthropometric Measures for CGDM Versus Controls

Child Anthropometric Measures	Total (n=52)	Control (n=27)	CGDM (n=25)	p[a]	CGDM Diet (n=17)	p[b]	CGDM Ins (n=8)	p[c]	p[d]
Weight* (kg)	34.9 (11.9)	34.9 (14.6)	36.4 (10.8)	0.56	34.5 (8.8)	0.82	40.8 (10.0)	0.19	0.12
Height (cm)	145.1±8.5	143.5±9.3	146.8±7.3	0.17	145.1±6.1	0.54	150.3±8.7	0.07	0.09
BMI Z Score	-0.19±1.0	-0.08±1.1	-0.30±0.9	0.45	-0.44±0.9	0.28	-0.01±0.9	0.86	0.26
Lean Body Mass* (kg)	25.1 (7.8)	25.1 (9.4)	25.6 (7.1)	0.54	25.1 (6.8)	0.81	28.8 (7.6)	0.22	0.24
Waist Circumference* (cm)	57.8 (7.8)	57.2 (13.7)	58.5 (6.8)	0.76	58.5 (5.4)	1.00	60.0 (9.8)	0.29	0.34
Waist to Height Ratio*	0.41 (0.03)	0.41 (0.08)	0.41 (0.03)	0.61	0.41 (0.02)	0.66	0.41 (0.06)	1.00	0.80
Triceps Skinfold* (mm)	11.7 (6.2)	12.8 (10.0)	11.0 (6.0)	0.28	10.7 (5.9)	0.27	12.5 (6.0)	0.89	0.44
Percent Body Fat*	31.2 (12.6)	32.9 (16.7)	29.4 (9.0)	0.18	27.9 (8.3)	0.20	31.6 (11.6)	0.69	0.55

Legend: BMI: body mass index; CGDM: children of mothers who had gestational diabetes; Ins: insulin. *Non-parametric; median (interquartile range) shown with the Mann-Whitney U-test for differences between groups. **P values:** a. CGDM vs Control; b. CGDM Diet vs Controls; c. CGDM Insulin vs Controls; d. CGDM Diet vs Insulin.

3.3.1. Primary Aim – GDM Status and CV Outcomes

Among primary outcomes, aPWV was higher in CGDM versus controls (p=0.03) suggesting greater aortic stiffness, driven largely by differences between CGDM whose mothers required insulin and controls (p=0.02) (Table 3.4). The difference between diet managed GDM and controls did not reach significance (p=0.30). There were no statistically significant differences in other measures including LV mass z score, lnRHI, pPWV and cIMT.

As for secondary outcomes (Table 3.5), the only measures that differed between CGDM and controls were the s' (p=0.03), e' (p=0.03), and a' (p=0.004) wave velocities, with higher velocities in CGDM compared to controls. CGDM of mothers with diet managed GDM also had significantly higher e' (p=0.04) and a' (p=0.02) wave velocities relative to controls. CGDM of mothers with insulin managed GDM only had significantly higher a' (p=0.02) wave velocities. These measures among CGDM and controls, however, were within normal limits [224,283,284]. IVSd z score was lower in children following insulin versus diet managed GDM (p<0.001), though neither differed from controls. None reached criteria for hypertrophy (z score >+2). In addition, mean arterial pressure (MAP) was higher in the CGDM insulin managed group relative to controls (p=0.04). There were no other statistically significant differences in secondary CV measures among the two GDM management groups and controls.

Table 3.4. Primary CV Outcomes in CGDM Versus Controls

CV Parameters	Total (n=52)	Control (n=27)	CGDM (n=25)	p[a]	CGDM Diet (n=17)	p[b]	CGDM Ins (n=8)	p[c]	p[d]
LVM Z Score	-0.19±0.61	-0.12±0.64	-0.25±0.58	0.46	-0.12±0.57	0.995	-0.52±0.51	0.12	0.11
lnRHI	0.28±0.33	0.30±0.31	0.26±0.36	0.62	0.27±0.33	0.76	0.21±0.46	0.54	0.72
aPWV (m/s)	5.5±2.2	4.8±1.6	6.2±2.6	0.03	5.5±2.2	0.30	7.9±2.6	0.02	0.03
pPWV (m/s)	6.8±0.6	6.8±0.6	6.7±0.7	0.45	6.7±0.8	0.44	6.8±0.4	0.74	0.78
cIMT (mm)	0.45±0.05	0.46±0.04	0.45±0.05	0.58	0.44±0.04	0.20	0.47±0.07	0.57	0.21

Legend: aPWV: aortic pulse wave velocity; CGDM: children of mothers who had gestational diabetes; cIMT: carotid intima-media thickness; CV: cardiovascular; Ins: insulin; lnRHI: logarithm transformed reactive hyperemia index; LVM: left ventricular mass; pPWV: peripheral pulse wave velocity.
P values: a. CGDM vs Control; b. CGDM Diet vs Controls; c. CGDM Insulin vs Controls; d. CGDM Diet vs Insulin

Table 3.5. Secondary CV Outcomes in CGDM Versus Controls

CV Parameters	Total	Control	CGDM	p[a]	CGDM Diet	p[b]	CGDM Ins	p[c]	p[d]
Secondary Outcomes	**(n=52)**	**(n=27)**	**(n=25)**		**(n=17)**		**(n=8)**		
SBP (mmHg)	104.3±7.4	104.2±8.0	104.5±6.8	0.89	103.6±7.1	0.79	106.4±6.2	0.48	0.34
DBP (mmHg)	66.0±5.5	64.9±5.5	67.2±5.4	0.12	66.6±6.3	0.33	68.5±2.4	0.08	0.42
MAP (mmHg)	78.8±5.5	78.0±5.6	79.7±5.4	0.27	78.9±6.2	0.59	81.2±2.8	0.04	0.35
PP (mmHg)	38.3±6.1	39.3±6.8	37.2±5.1	0.21	36.9±4.5	0.17	37.9±6.4	0.59	0.68
RHR (bpm)	75.0±8.8	76.1±8.8	73.7±8.9	0.34	72.3±8.0	0.15	77.1±10.7	0.79	0.24
Cardiac Wall Thickness									
IVSd Z Score	0.42±0.67	0.40±0.75	0.43±0.59	0.86	0.69±0.38	0.11	-0.11±0.62	0.09	<0.001
LVPWd Z Score	0.00±0.73	0.16±0.63	-0.16±0.79	0.12	-0.20±0.76	0.10	-0.07±0.91	0.43	0.71
Systolic Function									
s' Wave (cm/s)	8.9±1.4	8.5±1.6	9.3±1.1	0.03	9.3±1.1	0.07	9.4±1.3	0.14	0.82
SF (%)	34.8±5.7	33.8±5.2	35.7±6.2	0.24	35.5±7.4	0.38	36.1±2.7	0.24	0.83
LVSI Dopp (mL/m²)	36.7±7.0	37.3±6.0	36.0±8.0	0.49	37.4±6.8	0.96	32.9±9.9	0.26	0.20
LVCI Dopp (L/min/m²)	2.8±0.7	2.9±0.7	2.7±0.7	0.38	2.8±0.6	0.67	2.5±1.0	0.25	0.39
Diastolic Function									
E Wave (cm/s)	94.3±14.7	97.3±15.6	91.1±13.1	0.13	90.5±12.4	0.14	92.4±15.3	0.44	0.75
A Wave (cm/s)	45.9±10.3	47.4±11.9	44.3±8.2	0.28	43.5±7.9	0.20	46.0±8.9	0.76	0.49
e' Wave (cm/s)	16.8±2.9	16.0±3.0	17.7±2.5	0.03	17.8±2.5	0.04	17.4±2.8	0.23	0.73
a' Wave (cm/s)	6.0±1.2	5.5±1.3	6.5±0.9	0.004	6.4±1.0	0.02	6.7±0.9	0.02	0.43
MV DecT (ms)	150.6±28.6	155.6±25.5	145.2±31.3	0.19	147.2±29.6	0.33	140.9±36.3	0.20	0.65
MV E/A Ratio	2.2±0.5	2.2±0.5	2.1±0.5	0.86	2.1±0.4	0.83	2.2±0.8	0.96	0.93
MV A Dur (ms)	106.9±11.0	104.6±11.1	109.3±10.6	0.13	109.2±11.3	0.19	109.4±9.6	0.28	0.98
IVRT TDI (ms)	53.0±7.1	52.6±7.0	53.4±7.3	0.69	53.1±8.5	0.83	54.0±4.1	0.59	0.72

Global Function									
S/e'	0.53±0.07	0.54±0.07	0.53±0.07	0.91	0.53±0.06	0.65	0.55±0.09	0.66	0.48
Tei index TDI	0.43±0.06	0.42±0.05	0.43±0.07	0.91	0.42±0.07	0.81	0.44±0.08	0.63	0.55
Carotid Measures									
Compliance (mm²/kPa)	1.3±0.3	1.4±0.3	1.3±0.2	0.40	1.3±0.2	0.33	1.4±0.3	0.82	0.57
P-S Elast Mod (kPa)	33.6±7.6	33.4±8.7	33.9±6.3	0.83	34.5±6.5	0.68	32.8±6.3	0.87	0.57
β-Stiffness	3.0±0.8	3.0±0.9	3.0±0.6	0.95	3.1±0.6	0.76	2.9±0.5	0.58	0.31

Legend: CGDM: children of mothers who had gestational diabetes; CV: cardiovascular; DBP: diastolic blood pressure; Dopp: Doppler; Ins: insulin; IVRT: isovolumic relaxation time; IVSd: interventricular septal thickness in late diastole; LVCI: cardiac output indexed to body surface area; LVPWd: left ventricular posterior wall thickness in late diastole; LVSI: stroke volume indexed to body surface area; MAP: mean arterial pressure; MV A Dur: peak mitral valve flow velocity during atrial contraction; MV DecT: passive early filling wave deceleration time; MV E/A Ratio: passive early/atrial contraction peak velocities ratio; PP: pulse pressure; P-S Elast Mod: pressure-strain elasticity modulus; RHR: resting heart rate; S/e': peak systolic/passive early tissue Doppler velocities ratio; SBP: systolic blood pressure; SF: shortening fraction; TDI: tissue Doppler imaging. **P values:** a. GDM vs Control; b. GDM Diet vs Controls; c. GDM Insulin vs Controls; d. GDM Diet vs Insulin

3.3.1.1. The Influence of the Child's Age on Primary Outcomes

Due to the differences in age among groups, univariate linear models were built to investigate the associations of age with primary outcomes. As LV mass z score was calculated based on age- and sex-based normative curves, its relationship with age was not explored. The models revealed that age was not related to aPWV (B=0.17, p=0.73), pPWV (B=0.074, p=0.56), or cIMT (B=0.015, p=0.12); however, there was a positive linear association of age with lnRHI (B=0.15, p=0.03).

3.3.2. Potential Prenatal (Maternal) Mediators

3.3.2.1. Maternal Nutrition

In APrON surveys, all but three mothers in the present study reported consumption of prenatal multivitamins, with two of these three mothers reporting intake of general multivitamins and all three reporting folic acid supplementation.

Although reports in the second trimester were similar, mothers with pregnancies complicated by GDM reported consuming fewer less healthy foods in the third trimester of their pregnancies compared to controls (less healthy foods score, $p<0.001$). This same trend was apparent in both diet ($p=0.006$) and insulin managed GDM ($p=0.02$) versus controls. Further, while there was no evidence of overall dietary adjustment in controls from the second to third trimester of pregnancy with respect to CFG adherence and intake of less healthy foods, mothers diagnosed with GDM, particularly those with insulin managed GDM, showed evidence of dietary improvement (Table 3.6).

Table 3.6. Maternal Diet in Pregnancy

Maternal Diet Scores	2nd Trimester	3rd Trimester	Difference	p*
Control				
CFG (n=26)	3.0±1.2	3.5±2.0	0.42±2.08	0.31
LHS (n=26)	3.2±2.3	3.6±2.2	0.42±2.87	0.46
CFG-LHS	-0.15±2.57	-0.15±3.12	0.00±3.09	1.00
GDM				
CFG (n=21)	3.3±1.6	4.1±1.2	0.76±1.92	0.08
LHS (n=21)	3.4±2.5	1.7±1.5	-1.71±2.76	0.01
CFG-LHS	-0.14±2.65	2.33±1.93	2.48±3.50	0.004
GDM Diet				
CFG (n=15)	3.4±1.5	3.9±1.2	0.47±1.55	0.26
LHS (n=15)	3.1±2.2	1.7±1.5	-1.40±2.64	0.059
CFG-LHS	0.27±2.31	2.13±2.10	1.87±3.38	0.050
GDM Insulin				
CFG (n=6)	3.0±1.8	4.5±1.0	1.50±2.67	0.23
LHS (n=6)	4.2±3.1	1.7±1.5	-2.50±3.15	0.11
CFG-LHS	-1.17±3.37	2.83±1.47	4.00±3.63	0.04

Legend: CFG: Canada Food Guide score; GDM: gestational diabetes; LHS: less healthy foods score**.**
*Paired t-test

3.3.2.2. Maternal Iron Markers

Compared with controls, mothers with GDM had similar serum ferritin levels in the second trimester but significantly lower serum ferritin concentrations in the third trimester ($p=0.048$) (Table 3.7). Considering those with GDM, serum ferritin was lower in insulin managed GDM in the second trimester compared to diet managed GDM ($p=0.049$) and lower in diet managed GDM compared to controls in the third trimester ($p=0.02$). There were no statistically significant differences in other markers of iron status.

3.3.3. Potential Postnatal (Child) Mediators

3.3.3.1. The Diet of the Child

Forty-five children completed any dietary recalls: 28 completed all three, 13 completed two, and 4 completed one. Children's daily energy and macronutrient intakes are reported in Table 3.8. CGDM and controls did not differ in their overall energy and macronutrient intakes. Children of mothers with diet managed GDM did not differ from controls, while children of mothers with insulin managed GDM had a significantly higher carbohydrate intake relative to both controls ($p=0.04$) and diet managed cases ($p=0.01$). CGDM with maternal insulin treatment also had higher overall energy intake relative to those whose mothers were diet managed ($p=0.04$).

Table 3.7. Maternal Iron Markers in Pregnancy with Comparison Between GDM and Controls

Maternal Iron Markers	Total (n=52)	Control (n=27)	GDM (n=25)	p[a]	GDM Diet (n=17)	p[b]	GDM Ins (n=8)	p[c]	p[d]
Serum ferritin (ng/ml)									
2nd Trimester*	38.8 (39.2)	39.1 (46.1)	33.1 (38.5)	0.24	41.5 (39.5)	0.58	22.6 (28.1)	0.07	0.049
2nd Trimester Missing	13	10	3		1		2		
3rd Trimester*	13.7 (9.0)	17.4 (13.0)	12.7 (9.0)	0.048	11.6 (7.0)	0.02	14.1 (9.7)	0.59	0.26
3rd Trimester Missing	17	13	4		3		1		
TIBC (umol/L)									
2nd Trimester	71.8±9.0	69.4±8.6	73.7±9.0	0.14	73.4±9.0	0.21	74.3±9.8	0.23	0.84
2nd Trimester Missing	14	10	4		3		1		
3rd Trimester	86.2±12.7	82.8±8.4	89.1±15.1	0.14	88.9±12.5	0.12	89.3±20.2	0.27	0.96
3rd Trimester Missing	15	10	5		4		1		
Hemoglobin (g/L)									
2nd Trimester	121.1±9.7	122.7±11.1	119.3±7.7	0.25	119.6±8.0	0.34	118.7±7.7	0.42	0.82
2nd Trimester Missing	6	3	3		1		2		
3rd Trimester	120.3±9.6	120.1±11.9	120.5±6.6	0.91	120.4±7.4	0.94	120.6±5.6	0.92	0.96
3rd Trimester Missing	13	7	6		5		1		

Legend: GDM: gestational diabetes; Ins: insulin; TIBC: total iron binding capacity. *Non-parametric; median (interquartile range) shown with the Mann-Whitney U-test for differences between groups. **P values:** a. GDM vs Control; b. GDM Diet vs Controls; c. GDM Insulin vs Controls; d. GDM Diet vs Insulin

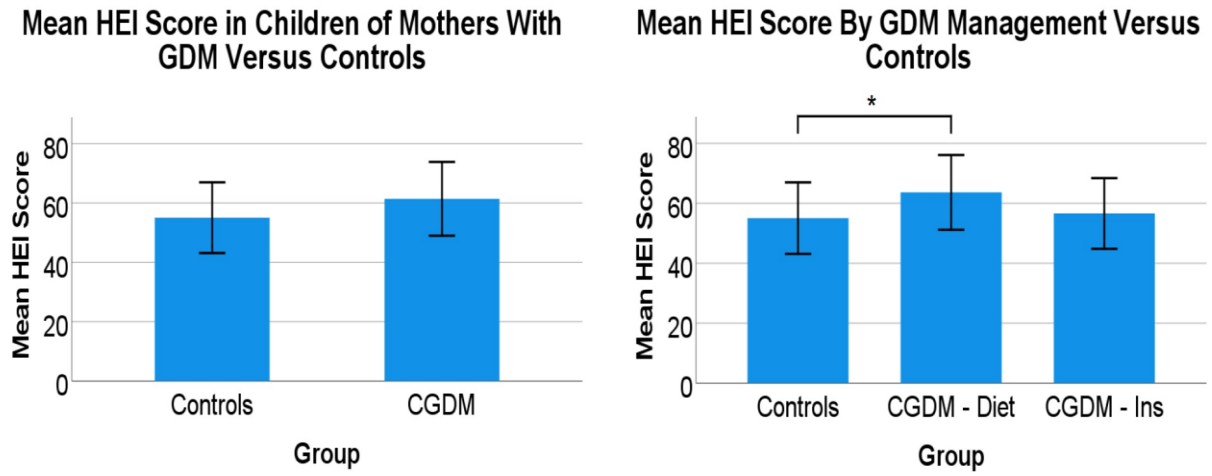
Table 3.8. Daily Energy and Macronutrient Intakes in CGDM Versus Controls

Child Daily Dietary Intake	**Total (n=45)**	**Control (n=23)**	**CGDM (n=22)**	**p[a]**	**CGDM Diet (n=15)**	**p[b]**	**CGDM Ins (n=7)**	**p[c]**	**p[d]**
Energy (kcal)	1867±469	1854±440	1881±508	0.85	1729±368	0.37	2207±637	0.11	0.04
Protein (g)	75.0±24.6	76.0±22.5	74.0±27.1	0.79	68.7±21.4	0.33	85.3±36.0	0.41	0.19
Fat (g)	68.7±24.6	69.0±25.7	68.4±24.0	0.93	63.4±21.5	0.49	79.0±27.5	0.38	0.16
Carbohydrate (g)	243.1±60.4	237.0±60.1	249.5±61.4	0.49	227.4±42.0	0.60	296.9±72.4	0.04	0.01

Legend: CGDM: children of mothers who had gestational diabetes; Ins: insulin. **P values:** a. CGDM vs Control; b. CGDM Diet vs Controls; c. CGDM Insulin vs Controls; d. CGDM Diet vs Insulin

While HEI scores were not significantly different between CGDM and controls or between children of mothers with insulin managed GDM versus controls, children whose mothers were in the diet managed group had significantly higher HEI scores than controls (63.7±12.5 vs 55.1±11.9, p=0.04) (Figure 3.1).

Figure 3.1. Comparisons of HEI Scores Between CGDM and Controls



Legend: CGDM: children of mothers who had gestational diabetes; GDM: gestational diabetes; HEI: healthy eating index; Ins: insulin. *p=0.04

3.3.3.2. Physical Activity of the Child

Of 51 children who wore ActiGraph accelerometers, valid data was obtained from 45. One of the participants was excluded from this analysis due to the child being ill during the week of wearing the accelerometer, while the others were excluded due to inadequate wear time. There were no statistically significant differences between groups in accelerometry-based measures of daily MVPA in CGDM versus controls (45.1±13.9 vs 49.6±17.6, p=0.36), or among diet or insulin controlled GDM groups (data now shown).

3.3.4. Impact of Potential Mediators on the Child's CV Health

Linear regression models for the relationship between potential pre and postnatal mediators and primary CV outcomes measures of the child identified several relationships (Tables 3.9 & 3.10a-d). With respect to the child's LV mass, univariate analyses revealed that higher percent body fat, lower HEI scores, and lower MVPA were related to lower LV mass z scores (Table 3.9). Upon building a multiple regression model with these three variables as covariates along with maternal GDM as a fourth covariate, the child's percent body fat and HEI score remained statistically significant (Table 3.10a). With regards to lnRHI, univariate analyses revealed that higher maternal serum ferritin in both the second and third trimesters (Figure 3.2) and the child's higher MVPA correlated with lower lnRHI scores (Table 3.9). Two separate multiple regression models were built to include mothers' second or third trimester serum ferritin and the child's age, sex, and MVPA. None of the parameters reached statistical significance (Tables 3.10b & 3.10c). Univariate analyses of factors contributing to aPWV revealed that a higher maternal CFG score in the third trimester, indicating a maternal diet in closer accordance with the CFG, was related to the child having a higher aPWV (Table 3.9). A multiple regression model was built including maternal GDM treatment mode versus controls, third trimester CFG score, and the child's age and sex. This model confirmed that maternal insulin managed but not diet managed GDM and CFG score in the third trimester had significant relationships with aPWV as previously noted (Table 3.10d). Finally, univariate regression analyses did not reveal any maternal or child factors contributing to pPWV or cIMT, and thus multivariate regressions were not used to explore these outcomes.

Table 3.9. Univariate Linear Models, Observing Maternal/Child Exposures and CV Outcomes

	LVM Z Score	lnRHI	aPWV (m/s)	pPWV (m/s)	cIMT (mm)
Maternal Variables					
Pre-preg. BMI (kg/m^2)	-0.029 (0.06)	-0.0045 (0.58)	0.0067 (0.90)	0.010 (0.48)	-0.00061 (0.60)
CFG 2nd Trim.	0.065 (0.31)	0.013 (0.70)	-0.016 (0.94)	-0.051 (0.43)	0.0045 (0.38)
CFG 3rd Trim.	-0.051 (0.33)	-0.033 (0.27)	0.50 (0.004)	0.022 (0.67)	0.0035 (0.39)
LHS 2nd Trim.	-0.034 (0.37)	-0.030 (0.13)	-0.099 (0.45)	0.025 (0.50)	0.0038 (0.20)
LHS 3rd Trim.	-0.0027 (0.95)	0.021 (0.36)	-0.11 (0.47)	-0.072 (0.08)	-0.00066 (0.84)
SF 2nd Trim. (ng/mL)	-0.00025 (0.94)	-0.0039 (0.01)	-0.012 (0.30)	0.0015 (0.63)	-0.00013 (0.59)
SF 3rd Trim. (ng/mL)	-0.0064 (0.34)	-0.011 (0.008)	0.010 (0.71)	0.0051 (0.46)	-0.0000029 (0.996)
TIBC 2nd Trim. (umol/L)	0.0047 (0.69)	0.011 (0.08)	0.0093 (0.83)	-0.0025 (0.83)	0.00073 (0.41)
TIBC 3rd Trim. (umol/L)	-0.00019 (0.98)	0.0052 (0.26)	-0.066 (0.057)	-0.0054 (0.53)	0.00070 (0.26)
Hemoglobin 2nd Trim. (g/L)	0.0050 (0.62)	0.0040 (0.44)	-0.046 (0.19)	0.0057 (0.56)	-0.00056 (0.44)
Hemoglobin 3rd Trim. (g/L)	-0.012 (0.26)	-0.0025 (0.67)	0.024 (0.52)	0.0036 (0.70)	-0.0016 (0.06)
Child Variables					
Percent Body Fat	-0.025 (0.004)	0.0089 (0.06)	0.0068 (0.84)	-0.0024 (0.79)	0.00034 (0.63)
HEI Score	0.023 (0.001)	0.00079 (0.85)	0.014 (0.62)	-0.012 (0.13)	-0.00037 (0.53)
MVPA Per Day (minutes)	0.013 (0.02)	-0.0062 (0.047)	-0.010 (0.62)	0.00016 (0.98)	-0.00041 (0.36)

Legend: Univariate regression coefficients and p values are shown. aPWV: aortic pulse wave velocity; BMI: body mass index; CFG: Canada Food Guide score; cIMT: carotid intima-media thickness; CV: cardiovascular; GDM: gestational diabetes; HEI: Healthy Eating Index; LHS: less healthy foods score; lnRHI: logarithm transformed reactive hyperemia index; LVM: left ventricular mass; Pre-preg. BMI: pre-pregnancy body mass index; MVPA: moderate to vigorous physical activity; pPWV: peripheral pulse wave velocity; SF: serum ferritin; TIBC: total iron binding capacity

Table 3.10a-d. Multiple Linear Models with Important Covariates

Table 3.10a. LV Mass Z Score Multiple Linear Model

	LV Mass Z Score (Adjusted R^2=0.40)	
Term	**B**	**p**
Constant	-0.29	0.60
Maternal GDM*	-0.23	0.17
Child's Percent Body Fat	-0.026	0.003
Child's HEI Score	0.020	0.008
Child's MVPA Per Day (minutes)	-0.00029	0.96

Legend: GDM: gestational diabetes; HEI: Healthy Eating Index; LV: left ventricular; MVPA: moderate to vigorous physical activity. *Relative to control group.

Table 3.10b. lnRHI Multiple Linear Model (2nd Trimester SF)

	lnRHI (Adjusted R^2=0.17)	
Term	**B**	**p**
Constant	-0.81	0.42
Maternal SF 2nd Trim. (ng/mL)	-0.0019	0.30
Child's Age (years)	0.11	0.19
Child's Sex – Female	0.22	0.13
Child's MVPA Per Day (minutes)	-0.0030	0.39

Legend: lnRHI: logarithm transformed reactive hyperemia index; MVPA: moderate to vigorous physical activity; SF: serum ferritin

Table 3.10c. lnRHI Multiple Linear Model (3rd Trimester SF)

	lnRHI (Adjusted R^2=0.12)	
Term	**B**	**p**
Constant	-0.48	0.69
Maternal SF 3rd Trim. (ng/mL)	-0.011	0.13
Child's Age (years)	0.090	0.34
Child's Sex – Female	0.17	0.35
Child's MVPA Per Day (minutes)	-0.0026	0.52

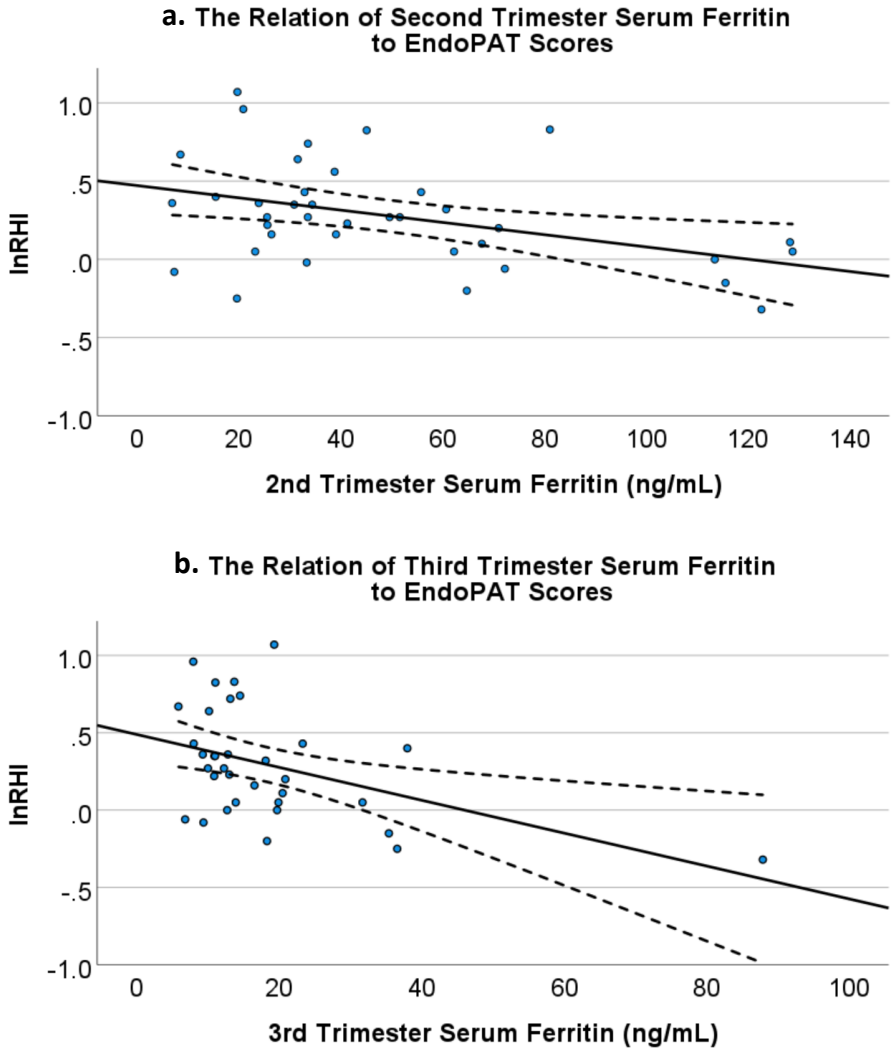
Legend: lnRHI: logarithm transformed reactive hyperemia index; MVPA: moderate to vigorous physical activity; SF: serum ferritin

Table 3.10d. Aortic PWV Multiple Linear Model

	aPWV (m/s) (Adjusted R^2=0.24)	
Term	**B**	**p**
Constant	9.5	0.051
Maternal GDM – Diet*	0.35	0.57
Maternal GDM – Ins*	2.8	0.005
Maternal CFG 3rd Trim. (ng/mL)	0.41	0.017
Child's Age (years)	-0.55	0.21
Child's Sex – Female	-0.22	0.74

Legend: aPWV: aortic pulse wave velocity; CFG: Canada Food Guide score; GDM: gestational diabetes; Ins: insulin. *Relative to control group.

Figure 3.2. Maternal Serum Ferritin in Pregnancy and the Child's EndoPAT Scores



Legend: Univariate regression line with 95% confidence limits. lnRHI: logarithm transformed reactive hyperemia index

3.4. Discussion

In this study of comparable groups of children in terms of other pre- and postnatal exposures, characteristics, and health behaviours, we found that children born to mothers who had GDM had increased aortic stiffness as indicated by higher aPWV, driven largely by CGDM whose mothers required insulin for glycemic control, in whom higher MAP was also observed.

These findings are in line with previous work from our group focused only on pregestational diabetes and aPWV measured in late infancy and early childhood [122,133] and preliminary work in preteens [261]. Interestingly, and in contrast to previous findings in children of mothers with pregestational diabetes [122,133,261], we found that CGDM in our study did not have increased LV mass relative to controls, and there were no statistically significant differences between CGDM and controls in other primary outcome measures including endothelial function, peripheral arterial stiffness, and cIMT. Secondary CV outcomes also revealed trends in regard to LV tissue and E and A wave blood flow velocities, which could be indicative of altered diastolic function and may contribute to an understanding of these findings. Together, these findings suggest in-utero exposure to GDM does have an impact on the vascular, and, to a lesser extent, myocardial health of the child.

3.4.1. Diabetes, Aortic Stiffness, and LV Mass

That vascular alterations are present in children following exposure to diabetes in pregnancy, both gestational and pregestational, is becoming increasingly apparent. Prior studies have investigated these changes from the fetal stages through infancy and childhood. Evanoff et al. [265] found that at 24-25 weeks gestation, fetuses of mother with diabetes (gestational and pregestational) had increased aortic compliance, indicative of early vascular changes. This finding added to an earlier observation from Hu et al. [285] that fetal aPWV increased in the third trimester in uncomplicated pregnancies, but not in pregnancies complicated by insulin-dependent diabetes. Although in the fetal stages the aorta seems to have greater elasticity in pregnancies complicated by diabetes, it appears that there is increased aortic stiffness postnatally, beginning in late infancy and continuing into adolescence [122,133,261]. Furthermore, Do et al. found that

aPWV in both late infancy [133] and at five years of age [122] positively correlated with maternal third trimester hemoglobin A1c, a measure of glycemic control in the preceding 3 months [136]. The present study adds to these findings, providing evidence that GDM also contributes to vascular changes in the children. That the increase in aortic stiffness was most pronounced in children of mothers who used insulin to manage their diabetes may also suggest a greater effect with worse glycemic control as previously noted by Do et al. [122,133]. On the other hand, it may indicate that there is a direct insult caused by maternal insulin therapy, as insulin has been shown to impact arterial stiffness in adults [286–288].

In a pilot study of preteen and teenage children exposed to maternal type 1 diabetes, aPWV and pPWV were both increased [261]. We did not find differences in pPWV between CGDM and controls in the present study. This indicates that perhaps there is a more severe effect of mothers having type 1 diabetes than GDM on the child's vascular health, perhaps relating to the earlier timing of the insult, already present in the 1st trimester. There may also be an important effect of age, as the children in the present study had a slightly younger mean age than those in this prior study. Upon investigation of the relation between age and both aPWV and pPWV, however, we found that there were no significant trends among our study participants. That there was a difference in aPWV but not pPWV in the present study indicates that structural differences in the aorta versus peripheral arteries, perhaps in the extracellular matrix, play an important role in the progressive stiffening of the arteries. The arteries near the heart, particularly the aorta, contain much more elastic tissue than more peripheral arteries [289]. Consequently, structural vascular changes leading to altered elasticity or stiffness of arteries may be more pronounced in the aorta. Peripheral arterial stiffness, on the other hand, is more dependent on the functional properties of the vessels, regulated by the sympathetic nervous system [290–292].

Therefore, differences in aPWV and pPWV likely represent different mechanisms of altered arterial stiffness.

It has long been recognized that maternal diabetes in pregnancy causes fetal myocardial hypertrophy [114–117]. A recent meta-analysis including 1925 mothers with diabetes and 2276 controls found that fetal myocardial hypertrophy was present in both GDM and pregestational diabetes, most notably in the third trimester, with altered diastolic function as indicated by E/A ratios in pregestational but not gestational diabetes [293]. Though many believed that this hypertrophy resolves in the early prenatal period, recent work at the University of Alberta demonstrated that it may persist for some into early childhood. In the latter study, young school aged children born to mothers with pregestational diabetes had increased indexed LV mass and increased IVSd z scores relative to controls [122]. Do et al. [122] postulated that perhaps this increased LV mass was a result of increased arterial/aortic stiffness in these children. In support of this, evidence in adults has shown that there is LV remodelling as indicated by increased wall thickness [135] and subclinical changes in LV strain [134] and twist mechanics [135] with greater arterial stiffness. Wewala et al. [261], however, found in older children (9-16 years) of pregestational mothers, despite increased aortic and peripheral arterial stiffness, no differences in LV wall thickness or mass were observed compared to controls. In the present study, we also did not find increased LV mass in children with fetal exposure to GDM. Other factors were more predictive of LV mass, including the child's anthropomorphic measures and overall dietary patterns suggesting postnatal factors may more importantly influence LV mass in children. Perhaps persistent LV hypertrophy in children of mothers with diabetes is exacerbated by increased arterial stiffness, but this hypertrophy resolves over time despite persistent arterial stiffness, at least early in life. This could suggest there are separate mechanisms contributing to LV

hypertrophy and arterial stiffness in children of mothers with diabetes. There may still be subclinical adverse remodeling, however, given subtle changes in ventricular filling. Further work is needed at this time to examine ventricular function using more sensitive measures including strain and twist mechanics which could elucidate subclinical changes.

3.4.2. Possible Mechanisms of CV Changes and Longer-term Risk

The adverse milieu associated with GDM includes hyperglycemia, hyperinsulinemia [294], and increased oxidative stress and reactive oxygen species [295], long implicated in increased CVD risk [296]. Increased LV mass in offspring of mothers with diabetes in pregnancy has been attributed to increased cardiomyocyte growth and proliferation due to hyperglycemia and hyperinsulinemia [117,297] and may result from increased expression of insulin-like growth factors [298,299], or it may result from similar mechanisms associated with adult diabetic cardiomyopathy, including impaired myocardial insulin signaling, metabolic stress, and oxidative stress and subsequent fibrosis and hypertrophy [300]. Moreover, additional evidence from murine models suggests that there are changes in the cardiac extracellular matrix, with increased collagen synthesis and deposition, in both fetuses [301,302] and adult offspring [303] as a result of in utero diabetes exposure. Adult rat offspring in the latter model were also found to have increased forkhead box protein O1 (FOXO1), a transcription factor with an important role in metabolism [304], and expression of its target genes, Mmp-2 and Ctgf, that contribute to extracellular matrix remodeling and increased myocardial collagen deposition [303].

Elastin and collagen are major structural proteins in the heart [305] and vascular wall [306]. While elastin provides recoil and gives arteries their elastic properties, collagen provides rigidity. In addition to its role in myocardial remodeling, increased collagen content may be an important

contributor to accelerated aortic stiffening in children of mothers with diabetes, as in vascular aging [307]. In fact, a rat model of protein restriction found both increased myocardial collagen and collagen I in aortas of adult rats following fetal growth restriction (FGR) [59], suggesting a common insult to the extracellular matrix in both the heart and aorta. The findings of the present study suggest that changes to the extracellular matrix, in the aorta and possibly in the heart, could be present in childhood and more persistent and therefore more important than LV hypertrophy in the etiology of long-term CVD in children of mothers with diabetes.

That previous studies have shown an increased aortic compliance [265] and lower aPWV [285] in utero with progressive stiffening after birth [122,133] gives insight into the timing of development of vascular stiffening but also raises the question as to the mechanisms of this progression. It appears that vascular development may be compromised in fetuses of mothers with diabetes and that there is then a compensatory stiffening of vessels postnatally. On the other hand, changes in early vascular structure during fetal development in pregnancies complicated by diabetes may be adaptive, but these changes then lead to progressive aortic stiffening postnatally. It is generally understood in adults and children that more compliant vessels are healthier [307,308], however, this may not be the case in the fetus. Progressive fetal vascular stiffening, which was found in uncomplicated pregnancies but not in pregnancies complicated by diabetes in a prior study [285], may be an indication of the healthy production and organization of elastin. Elastin is a very stable protein with a half-life of roughly 70 years [309]. It forms organized fibers in early fetal development and then functions throughout life to provide tissues with their elastic properties [310]. Synthesis of elastin peaks at around the time of birth in humans, continues through childhood and adolescence, and then declines thereafter [310,311]. Altered aortic elastin content has been shown in animal models of FGR from fetal stages [312–315]. A recent study in a rat model of

diabetes found no differences in elastin or collagen content of the thoracic aorta of adult offspring, but there were increased connections between arterial smooth muscle cells and the elastic lamellae [316]. It has previously been shown in elastin gene-knockout mouse models [317] and hypothesized in humans [318] that impaired elastin synthesis in the aorta early in life leads to increased vascular stiffness, and later development of hypertension. We propose that immature development, perhaps in elastin content or organization, of the fetal vascular extracellular matrix in the pregestational and gestational diabetic pregnancy leads to compensatory stiffening of the arteries, particularly the aorta, after birth, which may, in turn, contribute to longer-term CVD.

3.4.3. Other Pre- and Postnatal Mediators of Children's CV Health

GDM was associated with increased aortic stiffness in the children, particularly among those whose mothers used insulin for GDM management, increased MAP in children of mothers with insulin managed GDM, and altered tissue Doppler parameters of systolic and diastolic function. We have yet to understand the mechanisms associated with these changes. It does not appear, however, that these findings were related to other pre- or postnatal exposures that we assessed. The children in each group had comparable anthropometric measures, reported diets, and physical activity patterns, pointing to an effect of GDM rather than other possibly confounding factors. Upon examination of maternal dietary changes from the second to third trimester, we found that mothers with GDM made changes consistent with increased adherence to the CFG and decreased intake of less healthy foods. While this may seem to imply that there is little value in making dietary changes after GDM diagnosis, dietary changes are important in the management of GDM and likely contribute to better overall health and improved pregnancy

outcomes. There are many other factors beyond diet that may be responsible for the changes that were observed, such as maternal glycemic control or exposure to exogenous insulin.

Both univariate models and a multiple regression model with inclusion of relevant covariates revealed that a higher CFG score in the third trimester of pregnancy was associated with greater aortic stiffness in the child. This trend was the opposite of what we would have expected. However, insulin controlled GDM was also an important contributor to increased aPWV in the multiple regression model, and the effect of the mother's CFG score may have been driven by mothers with insulin controlled GDM having higher CFG scores in the third trimester relative to controls. As described here, it is difficult to tease out the confounding effect of maternal diabetes in the model.

Beyond the relationship between maternal diet and aPWV, both the child's percent body fat and HEI scores remained significant mediators of LV mass z score with inclusion of relevant covariates in the multiple regression model, with higher percent body fat and lower HEI score being associated with lower z scores. No other pre- or postnatal mediators remained statistically significant after selection of relevant variables for the multiple regression models, suggesting that these variables had relatively little impact on the children's CV health in this cohort.

3.4.4. Maternal Iron Status, GDM, and the Child's Endothelial Function

While serum ferritin levels were lower among GDM in the third trimester, higher serum ferritin in all mothers was associated with less flow-mediated vascular reactivity in children (Figure 3.2). Endothelial function, on the other hand, did not appear to be influenced by GDM exposure. The implications of these findings deserve further investigation, particularly since all serum ferritin values fell within or slightly below the normal range (12-150 ng/mL).

Iron is an essential mineral and plays many important roles in the body, including oxygen transfer, DNA synthesis, and electron transport [319]. Iron is also a very reactive free radical and generates reactive oxygen species in the body via the Fenton and Haber-Weiss reactions [320], causing oxidative stress when present in excess. Oxidative stress has been hypothesized to contribute to the development of GDM by inducing beta cell dysfunction, apoptosis, and decreased insulin secretion, as well as contributing to the development of insulin resistance in the liver and skeletal muscle [268–270]. Some investigators have suggested that systemic inflammation rather than increased iron stores may play a more important role in the development of GDM [155,321]. Some have gone so far as to suggest that iron supplementation may be harmful during pregnancy in the absence of iron-deficiency and thus should only be used when necessary [268,322,323], though this would need to be approached with caution due to the importance of iron in fetal growth and development, particularly in hemoglobin and myoglobin production and in neurodevelopment [324,325]. Iron balance, having neither too little nor too much, is clearly important for maternal and infant health and may have important implications for the child's CV health.

In the present study, we found that mothers with GDM did not have higher serum ferritin levels than controls in the second or third trimesters. In fact, maternal serum ferritin in the third trimester was lower in mothers with GDM than in controls. This finding was unexpected, but it may reflect a greater rate of iron transfer in pregnancies complicated by diabetes. Yang et al. [326] found that although maternal serum ferritin and hemoglobin did not differ in the late third trimester between women with and without GDM, females with GDM had higher soluble transferrin receptor levels with higher umbilical cord serum ferritin, indicating that iron is transported more actively in those with GDM than those without. This increased iron transfer

may reflect a greater need for iron in the fetus due to increased erythropoiesis driven by an increased metabolic rate and oxygen consumption in the presence of fetal hyperinsulinemia and hyperglycemia [327]. Despite this increased transfer, infants of mothers with diabetes are still at an increased risk for low iron stores [327].

Rat models have shown that in utero exposure to low maternal iron correlates with elevated systolic blood pressure in the adult offspring [272–274]. Data from the Avon Longitudinal Study of Parents and Children, however, revealed that maternal anemia in pregnancy, as indicated by hemoglobin levels, was not associated with elevated blood pressure in the children at three [328], seven [329], or ten [330] years of age. They did find that maternal iron intake seemed to have an important effect, though with mixed results as to the directionality of the effect [328–330].

The present study is the first to provide evidence for a link between maternal serum ferritin, a marker of maternal iron stores, and the offspring's endothelial function (Figure 3.2). Though this relation was attenuated in the adjusted models, the adjusted serum ferritin models were overall very weak. Importantly, reduced endothelial function in adults has been observed in the setting of high levels of serum iron [158,159]. Considering this finding along with results from the present study, we posit that higher iron stores in pregnancy, even within a normal range, may have a similar impact in the fetus. Though it has been shown that it may not be until iron levels are well above the normal range that adverse effects are seen in individuals, as in hereditary hemochromatosis [159], safe upper limits throughout the stages of pregnancy have not been established. Additional work is needed to verify the relationship between increased maternal serum ferritin and reduced endothelial function in the child. If this relationship is confirmed, it could have important implications for assessing and tracking serum ferritin levels and contributing factors such as inflammation and iron supplementation in pregnancy. It is not clear

from the present study whether the higher serum ferritin concentrations are related to inflammation (since ferritin is an acute phase protein), supplement intake, dietary intake, reduced transfer to the fetus at the time of the measurement, or a combination of these factors. More work is needed to understand the dynamics of iron metabolism in pregnancy [331].

3.4.5. Strengths and Limitations

This work represents a comprehensive assessment of early exposures, including data regarding the mother's and child's diet, metabolic status, and physical activity in particular, and their influence on various measures of the child's CV health. As a nested study, the APrON birth cohort provided detailed, prospectively gathered data collected during pregnancy. During their pregnancies, compared to provincial data, the mothers were older, had higher levels of education, and had higher household income than the general population [182]. Despite this possible bias, it is likely that a higher socioeconomic status is associated with better pre- and perinatal health behaviours, which may be protective against adverse effects of diabetes in pregnancy and could have contributed to the lack of differences in most of the child's CV measures. That we found differences even in this group, however, in such parameters as aPWV could imply differences may be more marked in the general population or suggest other exposures are less important in the evolution of vascular changes. As such, this may in fact represent a strength of this study.

Other limitations should be recognized. We reported LV mass estimations from one-dimensional images, using end-diastolic interventricular septal and LV posterior wall thickness and LV diameter, and adjusted for the children's lean body mass, which has recently been identified as an optimal predictor of LV mass in children [226]. However, there are many methods to calculated LV mass and z score. In the prior research examining children of mothers with

pregestational diabetes [122], LV mass was indexed by body surface area as recommended by the American Society of Echocardiography [223]. That similar trends of increased LV mass in children of mothers with diabetes were not found in our study may result from differences in the methods of adjustment, but it more likely suggests that body size and adiposity have a more important effect on LV dimensions as children age than prior exposure to maternal diabetes. It may also reflect differences related to the different types of diabetes.

The EndoPAT test represents a measure of endothelial function that has been shown to be predictive of dysfunction and CV risk in adults [251–253], it is automated, and thus provides a more objective, user-independent, assessment [249]. Despite evidence that this is a feasible [254] and a valid measure of endothelial function in children [255,256], one recent study suggested that the automated algorithms may not be optimized for use in children, perhaps missing the peak hyperemic response [332]. Gold standard measures involve infusion of endothelial-dependent vasodilators and imaging to measure changes in vessel diameters and are more invasive and time-intensive [250], thus not feasible in an observational study in children. The feasibility in children and user-independence of the EndoPAT test made it the test of choice for this study.

The echocardiographic assessments employed in this study require careful standardization to procure accurate and reliable measures. To minimize inter-operator variability, single, trained, study team members performed all cardiac (MEJM) and vascular (LKH) studies, following standard procedures. They were also blinded as to the diabetes status of the participants. Offline analyses of echocardiographic images for LV dimensions, systolic, and diastolic function parameters were completed following standard protocols, while vascular measures of aortic and peripheral PWV and cIMT were completed as described in the methods. Single study team members completed all of the measures for each parameter. One member

(JLM) completed all cIMT measures in blind while the remainder were completed by another (CB). As the latter researcher was involved in all stages of the research, including recruitment, establishment of data collection tools, scheduling of appointments, in-person assessments, and offline analysis of echocardiography images, complete blinding was not feasible. This represents a significant limitation of the study, particular with more user-dependent measures such as PWV and other echocardiography parameters.

Age and sex are well-established as important factors in CV programming. CGDM in this study, particularly those whose mothers were on insulin, were older than controls. It is unlikely, however, that this had an effect on our findings. In univariate models, age did not have a clear relationship with the primary outcomes, with the exception of vascular reactivity. This could perhaps have contributed to the lack of differences in lnRHI found between CGDM and controls. In the multiple linear models, age was not a significant contributing factor to the child's lnRHI score, nor did it contribute meaningfully to other models. It appears that age, for the most part, did not have an influence on the findings of the present study. Perhaps with a greater number of participants, the effects of age would become clearer. Besides age, there was a disproportionate representation of female participants in our study. Due to important differences in maturation and other sex-related differences, this may have impacted our findings. Perhaps a greater effect would have been seen in males, but we cannot support this hypothesis with the limited number of male participants in this study. Additional work with male offspring of mothers who had GDM would provide important information regarding sex-differences in CV outcomes following this exposure.

There are implications of other pre- and postnatal exposures that should be considered. This study was limited to those born at or near term and excluded any born with a birth weight

<5th percentile. It cannot be assumed the results would translate to those with other adverse intrauterine exposures. Additional work investigating infants born preterm or growth restricted to mothers with GDM would elucidate the effects of these additional adverse exposures on the health of the child in this context, as the risk of preterm birth and restricted fetal growth are elevated with maternal diabetes. Likewise, it becomes very difficult to tease apart the effects of different postnatal exposures when considering diet, exercise, and anthropometric measures, as these are often inter-related. However, groups were very comparable in measures of diet quality and physical activity, providing further support that the main findings of this study are valid.

Finally, this study had a relatively small sample size. While statistically significant differences were detected in some of the main outcomes of interest, the small sample size limited the regression analyses. A greater sample size would allow for greater refinement of the models and may detect effects that were missed due to large variation or insufficient power. In addition to sample size limitations, it is also important to consider the likelihood of type 1 errors with multiple comparisons.

3.4.6. Future Directions

Higher aPWV in children of mothers following pregnancies complicated by maternal GDM is a novel finding. More work is needed at this time to identify pathogenic mechanisms that explain this association. This may help to illuminate effective strategies to minimize the impact of maternal diabetes in pregnancy. Further, arterial stiffness in childhood has been identified as a predictor of later CVD [333]. Longitudinal studies tracing early aortic stiffness to later development of CVD would provide additional evidence that these vascular changes in

children following fetal exposure to maternal diabetes contribute to longer term risk of CVD in this cohort.

Additional physical activity, dietary, and CV analyses beyond what was included in this chapter may add to the findings of the present study. In addition to MVPA, we have access to additional information regarding the child's physical activity, including information about times spent being less active. Further analyses may reveal important trends. We have also collected detailed dietary information from the children including data regarding intake of vitamins, minerals, and other measures of food quality. Though beyond the scope of this project, further dietary analyses may reveal important relationships between dietary intake and CV outcomes.

Though we gained valuable information from the measures of CV health explored in this study, we did not complete a comprehensive assessment of CVD risk. Other data that would add to understanding the impact of GDM on the CV health of children in future studies include family history of CVD and hematological measures such as blood glucose, insulin, and lipid panels. The latter, in particular, were asked of participants but many declined this aspect of the study due to their invasive nature. Furthermore, there are more reliable and valid measures of preclinical cardiac dysfunction that were not employed in this study. Strain and rotational measurements may be more sensitive measures of pre-clinical systolic and diastolic dysfunction [334]. However, due to technical difficulties with image transfer and formatting, we have been unable as yet to complete strain assessments.

As a final note, there have recently been exciting advancement in diabetes management with closed loop systems. These systems utilize continuous glucose monitoring with an automated insulin pump and have been shown to optimize glycemic control in women with type 1 diabetes in pregnancy [335] and in individuals with type 2 diabetes [336]. This represents an exciting

advancement that may help to improve GDM management in the future. Future investigations of its efficacy in pregnancy should also include CV assessments in the child to examine its potential to improve outcomes.

3.5. Conclusions

In this study, we found that children of mothers who had GDM had increased aortic stiffness as indicated by higher aPWV, particularly those whose mothers required insulin in their pregnancies. This finding was not accompanied by increased LV mass. Higher maternal serum ferritin in pregnancy may be associated with worse endothelial function, a finding not influenced by maternal diabetes. Additional work should be done to understand the mechanisms of these changes and their implications for future CVD risk.

3.6. Acknowledgements

This research has been funded by the Stollery Children's Hospital Foundation and the Alberta Women's Health Foundation through the Women and Children's Health Research Institute. Study data were collected and managed using REDCap electronic data capture tools hosted at The University of Alberta [337,338]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Chapter 4: Surgical Outcomes in Infants with Major Congenital Heart Disease Exposed to Maternal Diabetes in Utero

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4.1. Introduction

The Developmental Origins of Health and Disease (DOHaD) model, credited to seminal work by David Barker [23,24], posits that exposures in the prenatal period set individuals on life-long health trajectories. The adverse effects of these exposures, particularly on cardiovascular (CV) health, may not become apparent until adulthood, although there could be evidence of subtle, though important, changes earlier in life.

Both fetal growth restriction (FGR) and preterm birth are associated with changes in cardiac structure and function in infancy and childhood that may have important consequences for adult cardiac function. FGR is associated with smaller, more globular hearts in infancy and throughout childhood, with altered left ventricular (LV) systolic and diastolic function [67–71,339]. Aortic diameters are also smaller in young adults with a history of FGR [340]. Barker's original observations discovered lower than normal birth weight to be associated with increased risk of death from cardiovascular disease (CVD) in adulthood [27,29], perhaps, at least in part, related to these CV alterations. With respect to preterm birth, a recent meta-analysis found a persistently decreased LV peak early diastolic tissue velocity and smaller LV end-diastolic dimension from infancy to early adulthood in affected infants, as well as decreased LV end-diastolic volume, stroke volume, and E/A ratio [260]. These early structural and functional changes may again contribute to increased risks later in life, with strong evidence from large national cohort studies

demonstrating increased heart failure [341] and ischemic heart disease [342] among adults with preterm birth. Studies in animal models have suggested these structural and functional changes may relate to altered cardiomyocyte structure and metabolism [343–346], changes in coronary reserve [347], and increased deposition and/or changes in the nature of myocardial collagen that contributes to diastolic dysfunction, in particular [74].

While for most affected infants and children such early adverse outcomes may not have a clinical or functional impact early in life, FGR and preterm birth as well as excessive or inadequate maternal weight gain in pregnancy have been identified as important risk factors for worse outcomes associated with congenital heart disease (CHD) surgery, particularly in infancy. This has been especially true for those requiring cardiopulmonary bypass (CPB) [169–173].

The factors that contribute to worse outcomes for CHD patients with early adverse exposures are not fully understood, although comorbidities contribute at least in part [348–351]. Rodent models, however, suggest another potential mechanism may relate to a worse myocardial response to ischemia-reperfusion that occurs with CPB. Ischemia followed by reperfusion after CPB is associated with ischemia-reperfusion injury (IRI) [352]. First recognized in pediatric CHD surgeries in the late 1980s and 1990s [175–178], IRI is characterized by myocardial inflammation and usually reversible damage resulting in reduced cardiac output in the first several hours to days postoperatively [353]. Rodent models of fetal hypoxia and nutrient restriction which mimic placental insufficiency and culminate in FGR [73,354] and of maternal diabetes [124,174] have demonstrated a greater reduction in heart function following 10-30 minutes of ischemia and subsequent reperfusion among affected offspring in young adulthood, with greater predilection in males. Additionally, the FGR models found increased myocardial collagen density in growth restricted offspring [354] as well as altered glucose metabolism post-ischemia [73]. Moreover, the

models of maternal diabetes found reduced baseline LV compliance [124] as well as impaired glucose tolerance and cardiomyocyte insulin signaling in offspring of diabetic dams, likely contributing to greater IRI [174]. The effects of adverse early exposures such as these on IRI have not yet been examined in humans at any age.

Similar to placental insufficiency with FGR, diabetes in pregnancy, which impacts approximately 1/6 pregnancies around the world [91], represents an adverse in utero exposure. The abnormal milieu of diabetes in pregnancy includes hyperglycemia and oxidative stress, particularly in cases of suboptimal glycemic control, and may include a need for insulin or other medication to maintain glycemic control. The three common types of diabetes involve insulin abnormalities through different mechanisms; type 2 diabetes and gestational diabetes (GDM) are associated with insulin resistance leading to hyperinsulinemia and beta cell dysfunction, while type 1 diabetes is charactered by beta cell destruction, necessitating insulin therapy. It has been well-established that diabetes exposure has adverse effects on the developing fetus including myocardial hypertrophy [293] that may persist long after birth [117,122,133] and altered arterial stiffness [122,133] as well as an increased risk long-term of CVD [108,109,111]. Whether exposure to a diabetic environment before birth contributes to worse surgical CHD outcomes in infancy, and whether this relates to greater IRI has not to date been examined.

The aim of this study was to investigate the surgical outcomes in infants with CHD born to mothers with diabetes undergoing repair at <1 year with comparison to infants with CHD born to healthy mothers. It was hypothesized that infants (IDM) of mothers with diabetes undergoing CPB have worse outcomes characterized by longer intensive care unit (ICU) and hospital length of stay (LOS) and that this could relate, at least in part, to greater IRI.

4.2. Methods

4.2.1. Study Design

This was a retrospective chart review study in which patients undergoing CPB surgery at the Stollery Children's Hospital in the first year of life, between 2008 and 2021, were identified through the Pediatric Cardiovascular Surgical and echocardiography databases, comparing outcomes between infants exposed and not exposed to maternal diabetes.

4.2.2. Patient Groups & Matching

IDM were identified and matched 1:2 with controls. Matching criteria included surgical intervention, age at surgery (during 1st month or within 6 weeks thereafter, where possible), biological sex, gestational age at birth (±3 weeks), being born small for gestational age (SGA; <10th centile), and with or without one of two common genetic syndromes (Trisomy 21 or 22q11.2 deletion syndrome), all of which have been shown to impact surgical outcomes [170,172,355–358]. In addition, maternal diabetes was separated into gestational or pregestational (Types 1 and 2) for additional sub-analyses.

4.2.3. Data Collection

Charts were reviewed and electronic medical record CV ICU data collected. Surgeries were coded according to a risk adjustment for congenital heart surgery (RACHS) scale to account for different levels of severity [356], and infants were separated into two groups of severity, RACHS A (less severe; groups 1-3) and RACHS B (more severe; groups 4-6).

Primary outcome variables included postoperative ICU and hospital LOS. Secondary outcomes comprised parameters associated with IRI, including Pediatric RISk of Mortality

(PRISM) scores [359], evidence of low output (including lowest mixed venous O_2 saturation and highest lactate in the first postoperative 48 hours), highest glucose in the first 48 hours, highest urea and creatinine, and length of ventilation.

4.2.4. Statistical Analysis

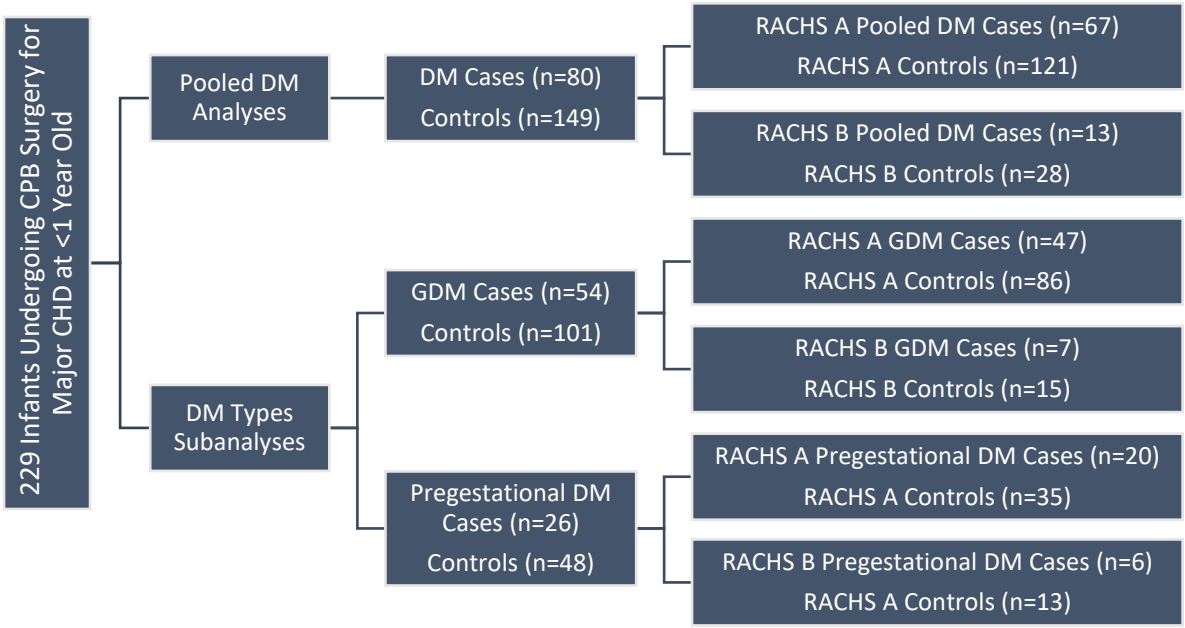
All data were described using means and standard deviations for numerical and proportions for categorical data. Statistical analyses included student's t-test or Pearson's chi squared test for parametric and Mann-Whitney U-test and Fisher's exact test for non-parametric data. P values of ≤ 0.05 were considered statistically significant, whereas those >0.05 and ≤ 0.15 were considered as nearing significance or showing possible trends.

Baseline variables, with diabetes both pooled and separated into pregestational and gestational, were described. Such variables included maternal age, gravidity, parity, multiple gestations, mode of delivery, infant's biological sex, gestational age at birth, birth weight, incidence of SGA births, and chromosomal abnormalities. In addition, surgical variables, including postnatal age at surgery, surgical era (2008-2012, 2013-2016, and 2017-2021), bypass time, and cross clamp time, were reported by RACHS group. For both primary and secondary aims, RACHS groups were analyzed separately, with outcomes of IDM compared with controls in each group. In the sub-analyses, cases were separated into gestational and pregestational diabetes exposure, with matched controls following the cases, and then analyzed by RACHS group as in the pooled analyses. Statistical analyses were completed using IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, N.Y., USA).

4.3. Results

Over the study period, 80 IDM and 149 controls with major CHD warranting surgical repair with the use of CPB were identified and included in the analysis. Of the 229 cases, 188 had surgical interventions categorized in RACHS pooled group A and 41 in RACHS pooled group B, with analyses completed as illustrated in Figure 4.1. Controls were matched to cases, as outlined in the methods, with a 1:2 case:control ratio in all but 11 instances. Specific CHD subgroups are presented in Table 4.1 with categorization by RACHS group.

Figure 4.1. Breakdown of Subjects into RACHS Groups for the Pooled Diabetes and Separate Analyses



Legend: RACHS Groups for the main analyses, with gestational and pregestational diabetes pooled, and for the sub-analyses, with gestational and pregestational diabetes and respective controls separated. Analyses were carried out between groups on the far-right side of the figure. CHD: congenital heart disease; CPB: cardiopulmonary bypass; DM: diabetes mellitus; GDM: gestational diabetes; RACHS: risk adjustment for congenital heart surgery

Table 4.1. CHD Categorized by RACHS Group

RACHS Group A (n=188)		
CHD	**RACHS Score**	**Incidence**
VSD	2	62
Single Ventricle	2	27
TOF	2	26
RVOTO	2	5
AP Window	2	2
AVSD	3	33
TGA	3	17
TOF with PA/Severe PS	3	10
VSD/Coarct	3	6
RACHS Group B (n=41)		
CHD	**RACHS Score**	**Incidence**
Truncus Arteriosus	4	11
Complex TGA	4	8
IAA	4	6
Complex Single Ventricle	4	2
Complex TOF	4	2
HLHS	6	12

Legend: AP Window: aorto-pulmonary window; AVSD: atrioventricular septal defect; CHD: congenital heart disease; Coarct: coarctation of the aorta; HLHS: hypoplastic left heart syndrome; IAA: interrupted aortic arch; PA: pulmonary atresia; PS: pulmonary stenosis; RACHS: risk adjustment for congenital heart surgery; RVOTO: right ventricular outflow tract obstruction; TGA: transposition of the great arteries; TOF: tetralogy of Fallot; VSD: ventricular septal defect

With respect to baseline maternal and infant variables, differences were only observed in maternal age, with older age among the mothers with diabetes, mode of delivery, with Caesarean section being more common for pregnancies complicated by diabetes (driven primarily by pregnancies complicated by pregestational diabetes), and gestational age at delivery, which was, on average, one week later among controls versus mothers with diabetes. There were non-significant trends towards higher gravidity among mothers with diabetes versus controls ($p=0.13$), particularly in the pregestational diabetes group ($p=0.080$), and lower birth weight among infants born to mothers with GDM ($p=0.10$), though this difference in birthweight was not apparent in the pooled diabetes or pregestational diabetes groups versus controls. There were no significant differences in maternal parity, twin/multiple pregnancy, biological sex of the infants, proportion born SGA, or with genetic syndromes (Table 4.2). As for surgical variables, there were no significant differences in any variables between RACHS groups, including postnatal age at surgery, surgical era/year, bypass, and cross-clamp time (Table 4.3). Baseline maternal/infant and surgical variables grouped by both type of diabetes and RACHS groups followed similar trends as above and are presented in Table 4.4a-d.

4.3.1. Main Pooled Diabetes Analyses

There were no statistically significant differences in ICU or hospital LOS or in parameters associated with IRI between IDM and controls in the pooled diabetes analysis and for each RACHS group (Table 4.5).

Table 4.2. Baseline Maternal and Infant Variables

Baseline Maternal and Infant Variables			
Variable	**Controls**	**IDM**	**p value**
Maternal Age (years)			
Pooled DM	29.8±6.1	32.9±5.6	<0.001
GDM	30.2±6.1	33.8±5.3	<0.001
Pregestational DM	28.8±6.2	31.2±5.8	0.11
Gravidity			
Pooled DM	2.6±1.7	3.2±3.4	0.13
GDM	2.7±1.8	3.2±4.0	0.50
Pregestational DM	2.5±1.6	3.2±1.9	0.080
Parity			
Pooled DM	1.1±1.3	1.3±1.5	0.49
GDM	1.1±1.3	1.3±1.5	0.53
Pregestational DM	1.2±1.4	1.3±1.5	0.73
Twins/Multiples			
Pooled DM	9/149 (6.0%)	4/80 (5.0%)	1.00
GDM	5/101 (5.0%)	3/54 (5.6%)	1.00
Pregestational DM	4/48 (8.3%)	1/26 (3.8%)	0.65
C-section Delivery			
Pooled DM	48/140 (34.3%)	38/77 (49.4%)	0.030
GDM	35/99 (35.4%)	20/53 (37.7%)	0.77
Pregestational DM	13/41 (31.7%)	18/24 (75.0%)	<0.001
Infant's Biological Sex - Female			
Pooled DM	83/149 (55.7%)	45/80 (56.3%)	0.94
GDM	61/101 (60.4%)	33/54 (61.1%)	0.93
Pregestational DM	22/48 (45.8%)	12/26 (46.2%)	0.98
Gestational Age at Birth (weeks)			
Pooled DM	38.0±1.7	37.1±2.0	<0.001
GDM	38.1±1.8	37.2±2.2	0.003
Pregestational DM	37.8±1.7	37.0±1.6	0.058
Birth Weight (kg)			
Pooled DM	3.01±0.58	2.95±0.75	0.56
GDM	2.96±0.61	2.79±0.71	0.10
Pregestational DM	3.11±0.51	3.30±0.72	0.23

SGA			
Pooled DM	16/149 (10.7%)	11/80 (13.8%)	0.50
GDM	15/101 (14.9%)	10/54 (18.5%)	0.55
Pregestational DM	1/48 (2.1%)	1/26 (3.8%)	1.00
Chromosomal Abnormality - T21			
Pooled DM	30/148 (20.3%)	17/79 (21.5%)	0.82
GDM	26/100 (26.0%)	15/54 (27.8%)	0.81
Pregestational DM	4/48 (8.3%)	2/25 (8.0%)	1.00
Chromosomal Abnormality - 22q11.2			
Pooled DM	6/148 (4.1%)	3/79 (3.8%)	1.00
GDM	3/100 (3.0%)	2/54 (3.7%)	1.00
Pregestational DM	3/48 (6.3%)	1/25 (4.0%)	1.00

Legend: Values are presented as mean±standard deviation for numerical variables or proportion (percentage) for categorical variables. 22q11.2: 22q11.2 (DiGeorge) syndrome; C-section: caesarean section; DM: diabetes mellitus; IDM: infants of mothers with diabetes; SGA: small for gestational age; T21: trisomy 21

Table 4.3. Baseline Surgical Variables

Baseline Surgical Variables			
Variable	**Controls**	**IDM**	**p**
Postnatal Age at Surgery (days)			
RACHS A	121.9±76.1	122.5±73.1	0.58
RACHS B	22.1±49.1	9.4±4.9	0.99
Surgical Era			
RACHS A			0.90
2008-2012	31/121 (25.6%)	17/67 (25.4%)	
2013-2016	61/121 (50.4%)	32/67 (47.8%)	
2017-2021	29/121 (24.0%)	18/67 (26.9%)	
RACHS B			0.75
2008-2012	7/28 (25.0%)	2/13 (15.4%)	
2013-2016	15/28 (53.6%)	7/13 (53.8%)	
2017-2021	6/28 (21.4%)	4/13 (30.8%)	
Bypass Time (minutes)			
RACHS A	87.9±32.7	85.5±35.2	0.67
RACHS B	149.5±51.1	150.4±43.8	0.96
Cross Clamp Time (minutes)			
RACHS A	52.6±30.2	48.4±29.9	0.41
RACHS B	81.1±37.7	83.9±24.3	0.82

Legend: Values are presented as mean±standard deviation for numerical variables or proportion (percentage) for categorical variables. IDM: infants of mothers with diabetes; RACHS: risk adjustment for congenital heart surgery

Table 4.4a-d. Baseline Variables by Types of Diabetes and RACHS Groups

Table 4.4a. Baseline Variables – GDM RACHS A

Variable	Controls (n=86)	IDM (n=47)	p
Maternal Age (years)	30.5±6.0	34.0±5.3	0.001
Gravida	2.7±1.9	3.5±4.2	0.30
Parity	1.1±1.3	1.4±1.6	0.42
Twins/Multiples	5/86 (5.8%)	3/47 (6.4%)	1.00
C-section Delivery	31/85 (36.5%)	18/47 (38.3%)	0.84
Infant's Biological Sex - Female	50/86 (58.1%)	28/47 (59.6%)	0.87
Gestational Age at Birth (weeks)	38.0±1.8	37.1±2.1	0.013
Birth Weight (kg)	2.92±0.61	2.78±0.71	0.23
SGA	14/86 (16.3%)	9/47 (19.1%)	0.68
Chromosomal Abnormality			
T21	26/85 (30.6%)	15/47 (31.9%)	0.87
22q11.2	1/85 (1.2%)	1/47 (2.1%)	1.00
SURGICAL VARIABLES:			
Postnatal Age at Surgery (days)	125.6±74.3	129.3±71.8	0.38
Surgical Era			0.77
2008-2012	19/86 (22.1%)	13/47 (27.7%)	
2013-2016	45/86 (52.3%)	23/47 (48.9%)	
2017-2021	22/86 (25.6%)	11/47 (23.4%)	
Bypass Time (minutes)	89.9±31.0	84.7±30.6	0.41
Cross Clamp Time (minutes)	55.7±29.0	48.6±25.7	0.21

Legend: Values are presented as mean±standard deviation for numerical variables or proportion (percentage) for categorical variables. 22q11.2: 22q11.2 (DiGeorge) syndrome; C-section: caesarean section; GDM: gestational diabetes; IDM: infants of mothers with diabetes; RACHS: risk adjustment for congenital heart surgery; SGA: small for gestational age; T21: trisomy 21

Table 4.4b. Baseline Variables – GDM RACHS B

Variable	Controls (n=15)	IDM (n=7)	p
Maternal Age (years)	28.5±6.2	32.1±5.0	0.19
Gravida	2.4±1.5	1.7±0.8	0.41
Parity	1.0±1.1	0.7±0.8	0.73
Twins/Multiples	N/A	N/A	N/A
C-section Delivery	4/14 (28.6%)	2/6 (33.3%)	1.00
Infant's Biological Sex - Female	11/15 (73.3%)	5/7 (71.4%)	1.00
Gestational Age at Birth (weeks)	38.6±1.5	37.7±2.6	0.32
Birth Weight (kg)	3.21±0.58	2.84±0.80	0.22
SGA	1/15 (6.7%)	1/7 (14.3%)	1.00
Chromosomal Abnormality			
T21	N/A	N/A	N/A
22q11.2	2/15 (13.3%)	1/7 (14.3%)	1.00
SURGICAL VARIABLES:			
Postnatal Age at Surgery (days)	24.1±60.6	7.9±3.2	0.95
Surgical Era			0.31
2008-2012	6/15 (40.0%)	1/7 (14.3%)	
2013-2016	7/15 (46.7%)	3/7 (42.9%)	
2017-2021	2/15 (13.3%)	3/7 (42.9%)	
Bypass Time (minutes)	145.9±59.6	148.7±21.3	0.92
Cross Clamp Time (minutes)	71.2±42.5	86.2±27.8	0.45

Legend: Values are presented as mean±standard deviation for numerical variables or proportion (percentage) for categorical variables. 22q11.2: 22q11.2 (DiGeorge) syndrome; C-section: caesarean section; GDM: gestational diabetes; IDM: infants of mothers with diabetes; RACHS: risk adjustment for congenital heart surgery; SGA: small for gestational age; T21: trisomy 21

Table 4.4c. Baseline Variables – Pregestational Diabetes RACHS A

Variable	Controls (n=35)	IDM (n=20)	p
Maternal Age (years)	28.8±6.0	32.2±5.4	0.046
Gravida	2.4±1.7	3.4±2.0	0.052
Parity	1.2±1.5	1.5±1.6	0.49
Twins/Multiples	3/35 (8.6%)	1/20 (5.0%)	1.00
C-section Delivery	10/29 (34.5%)	15/19 (78.9%)	0.003
Infant's Biological Sex - Female	16/35 (45.7%)	9/20 (45.0%)	0.96
Gestational Age at Birth (weeks)	37.7±1.9	36.7±1.5	0.055
Birth Weight (kg)	3.05±0.54	3.28±0.74	0.18
SGA	1/35 (2.9%)	1/20 (5.0%)	1.00
Chromosomal Abnormality			
T21	4/35 (11.4%)	2/20 (10.0%)	1.00
22q11.2	N/A	N/A	N/A
SURGICAL VARIABLES:			
Postnatal Age at Surgery (days)	112.9±80.7	106.6±75.4	0.80
Surgical Era			0.36
2008-2012	12/35 (34.3%)	4/20 (20.0%)	
2013-2016	16/35 (45.7%)	9/20 (45.0%)	
2017-2021	7/35 (20.0%)	7/20 (35.0%)	
Bypass Time (minutes)	83.0±36.7	87.1±44.7	0.73
Cross Clamp Time (minutes)	44.9±32.2	48.0±38.9	0.77

Legend: Values are presented as mean±standard deviation for numerical variables or proportion (percentage) for categorical variables. 22q11.2: 22q11.2 (DiGeorge) syndrome; C-section: caesarean section; IDM: infants of mothers with diabetes; RACHS: risk adjustment for congenital heart surgery; SGA: small for gestational age; T21: trisomy 21

Table 4.4d. Baseline Variables – Pregestational Diabetes RACHS B

Variable	Controls (n=13)	IDM (n=6)	p
Maternal Age (years)	28.7±7.1	28.2±6.6	0.88
Gravida	2.6±1.1	2.5±1.0	0.97
Parity	1.2±1.1	0.8±1.0	0.64
Twins/Multiples	1/13 (7.7%)	0/6 (0%)	1.00
C-section Delivery	3/12 (25.0%)	3/5 (60.0%)	0.28
Infant's Biological Sex - Female	6/13 (46.2%)	3/6 (50.0%)	1.00
Gestational Age at Birth (weeks)	38.2±0.8	38.2±1.7	0.93
Birth Weight (kg)	3.27±0.37	3.38±0.74	0.68
SGA	N/A	N/A	N/A
Chromosomal Abnormality			
T21	N/A	N/A	N/A
22q11.2	3/13 (23.1%)	1/5 (20.0%)	1.00
SURGICAL VARIABLES:			
Postnatal Age at Surgery (days)	19.7±33.5	11.2±6.1	0.90
Surgical Era			1.00
2008-2012	1/13 (7.7%)	1/6 (16.7%)	
2013-2016	8/13 (61.5%)	4/6 (66.7%)	
2017-2021	4/13 (30.8%)	1/6 (16.7%)	
Bypass Time (minutes)	153.2±43.2	152.4±65.0	0.98
Cross Clamp Time (minutes)	91.0±30.8	81.2±22.2	0.53

Legend: Values are presented as mean±standard deviation for numerical variables or proportion (percentage) for categorical variables. 22q11.2: 22q11.2 (DiGeorge) syndrome; C-section: caesarean section; IDM: infants of mothers with diabetes; RACHS: risk adjustment for congenital heart surgery; SGA: small for gestational age; T21: trisomy 21

Table 4.5. Surgical Outcomes by RACHS Groups – Pooled Diabetes

RACHS Group A – Pooled DM			
Outcomes	**Controls**	**IDM**	**p**
ICU LOS (days)	3.6±3.0	3.9±5.4	0.17
Hosp LOS (days)	9.9±7.9	12.1±17.9	0.50
EVIDENCE OF IRI			
PRISM Score*	7.6±4.8	7.6±4.3	1.00
Lowest MV O_2 sat* (%)	48.9±12.3	49.1±12.7	0.91
Highest lactate* (mmol/L)	3.0±1.7	2.9±1.2	0.91
Highest glucose* (mmol/L)	12.0±4.1	12.2±3.3	0.23
Highest urea (mmol/L)	8.1±4.1	9.0±4.3	0.19
Highest creatinine (µmol/L)	46.7±16.0	44.9±13.2	0.68
Days intubated	3.1±3.2	2.7±2.8	0.53
RACHS Group B – Pooled DM			
Outcomes	**Controls**	**IDM**	**p**
ICU LOS (days)	5.6±3.6	5.2±2.6	0.97
Hosp LOS (days)	24.6±18.5	24.0±16.5	0.97
EVIDENCE OF IRI			
PRISM Score*	11.3±4.6	13.2±6.2	0.30
Lowest MV O_2 sat* (%)	41.4±13.8	43.4±9.1	0.66
Highest lactate* (mmol/L)	6.2±3.3	5.9±1.8	0.87
Highest glucose* (mmol/L)	15.6±3.1	14.8±2.4	0.44
Highest urea (mmol/L)	14.2±8.5	14.2±10.0	0.76
Highest creatinine (µmol/L)	64.3±29.6	95.5±117.9	0.65
Days intubated	8.2±6.1	9.3±4.8	0.19

Legend: DM: diabetes mellitus; Hosp: hospital; ICU: intensive care unit; IDM: infants of mothers with diabetes; IRI: ischemia-reperfusion injury; LOS: length of stay; MV: mixed venous; PRISM: **P**ediatric **RIS**k of **M**ortality; RACHS: risk adjustment for congenital heart surgery.
*First 24 to 48 hours

4.3.2. Diabetes Subgroups Analyses

In the separate analyses of gestational and pregestational diabetes, IDM exposed to GDM exhibited some evidence of better outcomes (Table 4.6) while those exposed to pregestational diabetes showed some evidence of worse outcomes (Table 4.7). With respect to primary and secondary outcomes, IDM exposed to GDM were in the ICU for one day less than controls on average in RACHS Group A (p=0.022), and those in RACHS Group B had lower glucose levels than controls postoperatively (13.4 vs 16.7, p=0.014). There were no other trends or differences in outcomes among groups in the GDM comparisons.

When comparing infants of mothers with pregestational diabetes to controls, IDM in RACHS Group A had a longer hospital LOS (10 days longer on average, though with greater variability (p=0.046). The magnitude of this difference was largely driven by a single complex case. When this case was removed, the difference was less marked and no longer statistically significant, though still nearing significance (13.1 vs 9.8 days, p=0.082). A few secondary variables differed in IDM exposed to pregestational diabetes compared to controls. In RACHS Group A, IDM exhibited higher postoperative urea (p=0.035). Mean PRISM scores in Group B IDM were more than 50% higher than controls (15.6 vs 10.0), a difference with a trend towards significance (p=0.11). Again in Group B IDM, there were signs of increased postoperative glucose relative to controls (nearing significance; 16.7 vs 14.3, p=0.059). Creatinine (136.4 vs 57.2, p=0.50) levels were also higher in Group B IDM, though these differences did not reach statistical significance nor show trends towards significance, likely due to the large variability among IDM.

Table 4.6. Surgical Outcomes by RACHS Groups – GDM Only

RACHS A - GDM			
Outcomes	**Controls (n=86)**	**IDM (n=47)**	**p**
ICU LOS (days)	3.8±3.1	3.0±2.6	0.022
Hosp LOS (days)	10.0±8.4	8.9±7.8	0.63
EVIDENCE OF IRI			
PRISM Score*	8.0±4.9	8.2±4.5	0.84
Lowest MV O_2 sat* (%)	48.0±12.6	49.0±12.7	0.68
Highest lactate* (mmol/L)	3.1±1.9	2.8±1.3	0.39
Highest glucose* (mmol/L)	11.9±4.0	12.1±3.4	0.40
Highest urea (mmol/L)	8.0±4.1	8.2±4.1	0.81
Highest creatinine (µmol/L)	46.9±17.2	43.5±12.8	0.50
Days intubated	3.3±3.4	2.3±1.8	0.18
RACHS B – GDM			
Outcomes	**Controls (n=15)**	**IDM (n=7)**	**p**
ICU LOS (days)	5.9±4.0	5.3±2.2	1.00
Hosp LOS (days)	18.4±12.6	23.3±11.3	0.34
EVIDENCE OF IRI			
PRISM Score*	12.3±3.8	11.2±2.2	0.50
Lowest MV O_2 sat* (%)	40.4±12.7	42.3±9.2	0.74
Highest lactate* (mmol/L)	6.8±3.8	6.0±1.8	0.84
Highest glucose* (mmol/L)	16.7±3.0	13.4±2.0	0.014
Highest urea (mmol/L)	14.7±7.5	11.3±4.5	0.27
Highest creatinine (µmol/L)	70.4±30.2	66.3±23.5	0.84
Days intubated	6.9±4.3	8.0±1.9	0.16

Legend: GDM: gestational diabetes; Hosp: hospital; ICU: intensive care unit; IDM: infants of mothers with diabetes; IRI: ischemia-reperfusion injury; LOS: length of stay; MV: mixed venous; PRISM: **P**ediatric **RIS**k of **M**ortality; RACHS: risk adjustment for congenital heart surgery
*First 24 to 48 hours

Table 4.7. Surgical Outcomes by RACHS Groups – Pregestational Diabetes Only

RACHS A - Pregestational DM			
Outcomes	**Controls (n=35)**	**IDM (n=20)**	**p**
ICU LOS (days)	3.1±2.5	5.9±8.7	0.39
Hosp LOS (days)	9.8±6.5	19.5±29.3	0.046
EVIDENCE OF IRI			
PRISM Score*	6.9±4.4	6.5±3.6	0.78
Lowest MV O_2 sat* (%)	51.0±11.3	49.4±13.1	0.65
Highest lactate* (mmol/L)	2.7±1.3	3.0±1.2	0.19
Highest glucose* (mmol/L)	12.2±4.4	12.5±3.2	0.52
Highest urea (mmol/L)	8.4±4.2	11.0±4.4	0.035
Highest creatinine (µmol/L)	46.4±13.0	48.2±13.9	0.61
Days intubated	2.7±2.4	3.9±4.3	0.36
RACHS B - Pregestational DM			
Outcomes	**Controls (n=13)**	**IDM (n=6)**	**p**
ICU LOS (days)	5.3±3.3	5.0±3.2	0.90
Hosp LOS (days)	31.9±22.0	24.7±21.7	0.37
EVIDENCE OF IRI			
PRISM Score*	10.0±5.3	15.6±8.8	0.11
Lowest MV O_2 sat* (%)	42.3±15.2	45.0±9.8	0.72
Highest lactate* (mmol/L)	5.4±2.6	5.7±1.9	0.78
Highest glucose* (mmol/L)	14.3±2.7	16.7±1.3	0.059
Highest urea (mmol/L)	13.6±9.8	18.1±14.6	0.50
Highest creatinine (µmol/L)	57.2±28.5	136.4±183.9	0.50
Days intubated	9.8±7.6	11.0±7.2	0.63

Legend: DM: diabetes mellitus; Hosp: hospital; ICU: intensive care unit; IDM: infants of mothers with diabetes; IRI: ischemia-reperfusion injury; LOS: length of stay; MV: mixed venous; PRISM: **P**ediatric **RIS**k of **M**ortality; RACHS: risk adjustment for congenital heart surgery
*First 24 to 48 hours

4.4. Discussion

This study is the first to explore the impact of an intrauterine exposure to maternal diabetes on the clinical outcomes of infants undergoing surgery for major CHD. As such, this work is largely exploratory in nature. Taken together, findings from the main pooled and separate diabetes analyses provide some evidence that compared to infants born to mothers with healthy pregnancies, those exposed to pregestational diabetes may have worse outcomes. Despite limited numbers, those in RACHS Group A exhibited longer hospital stays and higher postoperative urea, and those in RACHS Group B tended to have higher PRISM scores and postoperative glucose relative to controls, albeit not reaching statistical significance, findings that could reflect greater IRI. Interestingly, and in contrast, we found IDM exposed to GDM to have better outcomes, with shorter ICU stays (RACHS Group A) and lower postoperative glucose (RACHS Group B), findings that were unexpected. The lack of statistically significant differences in the pooled diabetes analyses by RACHS groups could have reflected the differing levels of impact of gestational and pregestational diabetes, with results being disproportionately influenced by infants exposed to GDM, representing two-thirds of the entire cohort examined.

While the etiologies differ, the different types of diabetes have common features, characterized by hyperglycemia and often requiring medication, most commonly insulin, for management. Diabetes in general is known to be an inflammatory condition [360–363], and this inflammation may contribute to the development of adverse CV outcomes and CVD in mothers with diabetes and their offspring [364]. These similarities led us to believe that there may be a common insult in pregnancies complicated by diabetes potentially leading to greater IRI and worse outcomes among prenatally exposed infants. However, the findings of our study suggest a divergence in the influence of pregestational versus gestational diabetes and/or the presence of

other contributing factors that impact surgical outcomes of affected infants. Unique aspects of each intrauterine milieu, not the least of which include differences in length of intrauterine exposure and in developmental stages of exposure, could have contributed to these findings. Furthermore, discovery of GDM in the mid-trimester could have led to greater awareness of the woman's health, thus resulting in intentional improvement in diet, and this could have translated to optimized care of their child.

To date, previous work exploring the effects of fetal exposure to maternal diabetes on cardiac recovery after ischemia-reperfusion has only been completed in adult animal models [124,174]. In these models, the added insults of aging and inactivity (due to being caged) could have contributed to greater IRI in offspring exposed to diabetes in utero. The important contribution of postnatal exposures has been suggested from animal models exploring the impact of adverse prenatal exposures on adult CV health [125,162]. If in fact maternal diabetes, particularly pregestational, exposure truly increases myocardial risks of IRI in humans, the lack of more significant differences in our cohort could relate to the early ages explored and/or more subtle abnormalities that may only be elucidated through a larger study. Additionally, animal models do not perfectly mirror clinical conditions, and those mimicking diabetes in pregnancy, are no exception.

4.4.1. Confounding Variables

There are several important variables whose influence should be considered in discussing these findings. Maternal diabetes is known to be associated with increased risk of preterm birth, Caesarean section, and being born SGA. These adverse early conditions alone may contribute to adverse outcomes in IDM.

Gestational age at birth was significantly different between groups in the present study, with IDM born at 37 weeks and controls at 38 weeks on average. The earlier deliveries in pregnancies complicated by diabetes may have represented care-related choices. Costello et al. [170] and Steurer et al. [365], demonstrated in large cohorts with CHD that birth above 39 weeks was associated with the lowest in-hospital mortality and morbidity. In contrast, even those at 37-38 weeks demonstrated worse outcomes. Thus, just these slight differences in gestational age could have contributed to differences observed. Caesarean section represents another possible confounding variable. Caesarean section, more common among mothers with diabetes, as also witnessed in the present study, is known to be associated with adverse outcomes for both mothers and children [366]. Not only was Caesarean section associated with earlier birth, but it was also associated with increased hospital LOS in infants undergoing surgery for CHD in a study by Peyvandi et al. [367]. In our pooled diabetes versus controls analyses, that we did not demonstrate differences between IDM and controls was again due to either our relatively small cohort or impacted by the improved outcomes for infants of mothers with GDM. Future studies should again involve both larger cohorts and either attempt to control for gestational age at birth and birth mode or explore further their relative impact.

4.4.2. Additional Limitations & Future Directions

It is important to acknowledge some additional limitations to this study. We chose to categorize infants according to RACHS scores to provide sufficient numbers of IDM for us to conduct robust statistical analyses; however, this resulted in increased variability within RACHS groups. Still, with limited numbers studied, this approach provided groupings of infants with comparable severity of CHD and surgical risks, including length of cardiopulmonary bypass and

circulatory arrest, insults that contribute to worse IRI. It is limited, however, in that it cannot measure confounding variables including the nuances of CHD in a given child or unexpected intraoperative occurrences, including other insults.

Our study, as alluded to above, was limited by small patient numbers, particularly when exploring diabetes and RACHS subtypes. Our observations, however, could inform the organization of larger multicenter studies, defining the necessary numbers to be included to potentially achieve statistical significance particularly in variables that tended to demonstrate differences. It is important to take into account the possibility of chance findings when considering the differences noted in the separate analysis of gestational and pregestational diabetes. However, the small patient numbers in this dataset, particularly among pregestational diabetes groups, would have been more likely to lead to type 2 errors, failing to reject the null hypothesis when it is false. That there were some significant differences, other findings that tended to demonstrate a difference, and still others that showed quite divergent means but did not reach even a trend at this time highlights the need again for a much larger cohort of infants to investigate possible differences and further exploration of the mechanisms underpinning differences between diabetes subtypes. Given the findings reported from animal models used to explore the impact of adverse prenatal exposures on postnatal CV health [65,66,72,73,124], biological sex is likely another important contributing factor that should be explored in a larger cohort.

While hospital and ICU LOS represent measures that may be indicative of time needed to recover from surgery, the secondary measures of IRI used in this study may not have given a full picture of the extent of IRI. Additionally, any one of the primary and secondary parameters examined could have been influenced by insults and other confounding variables beyond IRI. Despite the latter limitation, they still have value in understanding the impact of maternal

diabetes and, taken together, provide a more reliable assessment of the extent of the insult than any one of the measures alone. Future studies could be designed to investigate additional measures of IRI such as reduced ejection fraction assessed by echocardiography and need for inotropes [353]. Although intraoperative and postoperative echocardiograms are available for this cohort, data regarding inotropes were not included in the present study due to difficulty acquiring complete data.

4.5. Conclusions

Exposure to the intrauterine milieu associated with maternal diabetes is known to negatively impact CV health very early in life and long-term. Our study explored for the first time its influence on operative outcomes associated with major CHD requiring CPB in the first year. Despite small numbers, the findings could suggest a divergence in outcomes associated with exposure to gestational versus pregestational diabetes, with better outcome measures in the former and worse in the latter. Whether these findings relate to differences in susceptibility to IRI or not is not clear. These preliminary results call for additional work with a greater number of affected infants, particularly those exposed to pregestational diabetes, which could be informed by the present work. Ultimately, if a link is clearly established between maternal diabetes and CHD outcomes, exploration of contributing mechanisms could lead to novel approaches to mitigating risks.

Chapter 5: Overview of Findings and Future Directions

5.1. The Impact of Gestational Diabetes on the Cardiovascular Health of the Child

5.1.1. Summary of Findings

Our findings suggest the presence of early cardiovascular (CV) changes in children of mothers with gestational diabetes (GDM). They further potentially shed light on at least one mechanism whereby this adverse prenatal exposure could contribute to longer-term CV disease (CVD) in affected offspring. We found increased aortic stiffness, suggested by increased pulse wave velocities, in children with in-utero exposure to GDM (6.2±2.6 vs 4.8±1.6 m/s, p=0.03), and this was most apparent in those whose mothers required insulin therapy (7.9±2.6 m/s, p=0.02 versus controls). Arterial stiffness of the aorta has been established as an indicator of CV health in adulthood, predicting CV morbidity and mortality [368], and is understood to contribute to adverse left ventricular (LV) remodeling [61,134,135]. Arterial stiffness has also been linked to the development of hypertension [12]. Thus, increased aortic stiffness in childhood following in utero exposure to GDM could very well contribute to the evolution of CVD.

Our findings elucidate the importance of timing of the insult that contributes to vascular changes. GDM is first diagnosed in the mid-trimester, between 24 to 28 weeks [187]. That greater aortic stiffness was observed despite improvements in maternal diet quality in later gestation suggests either again the mid-trimester timing of exposure is critical and/or other factors beyond diet are at play. Our results further highlight the need for additional research in this area examining the complex relationships among CV outcomes in the child and the timing and practical aspects of implementing therapies aimed at controlling diabetes in pregnancy. For example, initiating insulin and/or diet therapy at mid-pregnancy may be too late or insufficient to

improve aortic stiffness, yet these therapies may still have a positive impact on the mother's and infant's health not considered in the current study. Earlier screening for GDM and/or exploration of other contributing factors associated with GDM evolution may be necessary to provide a full understanding of the relationship between maternal GDM and changes in the aortic composition of the child.

We found the child's percent body fat, diet quality, and daily amount of moderate to vigorous physical activity to not obviously influence aortic stiffness. This suggests that at least in the case of aortic stiffness, in-utero insults have lasting effects that persist despite the child's positive health behaviours. While there was a lack of a relationship with aortic stiffness in our study, the importance of healthy diet and physical activity in long-term CV health is well-established and may have other benefits that ultimately mitigate longer term effects of increased aortic stiffness. As an example, such behaviors are important in maintaining metabolic homeostasis that when altered, as occurs in metabolic syndrome, can have a detrimental impact on vascular health long-term [369].

Beyond aortic stiffness, we found a lack of differences in other measures of CV structure and function. In particular, we found that LV mass normalized to lean body mass was not increased following in-utero exposure to GDM (z score of -0.25±0.58 in children of mothers who had GDM versus -0.12±0.64 in controls, p=0.46). This finding was in contrast with previous findings of increased LV mass in infants and children aged 5 years following exposure to maternal pregestational diabetes [122,133]; however, another pilot study in older children (9-16 years) with in utero exposure to maternal pregestational diabetes also found that despite increased arterial stiffness, LV mass and dimensions were not different between exposed and unexposed children [261]. While increased arterial stiffness has been shown to contribute directly to

changes in LV composition and function [61,134,135], differences in LV mass and dimensions in children of mothers with GDM versus controls were not apparent in the present study. The increased aortic stiffness observed may not have been of sufficient duration to result in clinically measurable differences in the children. Furthermore, more subtle but clinically measurable changes may require the evaluation of a larger cohort. Assessment of adolescents and young adults through cross-sectional studies and/or the longitudinal evaluation of the current cohort may be necessary. Furthermore, strain analyses and exploration of LV rotational mechanics may be necessary to elucidate more subtle myocardial functional changes reflective of altered LV afterload.

Our study discovered maternal serum ferritin levels in the second and third trimester to negatively correlate with the child's EndoPAT scores (B=-0.0039, p=0.01 and B=-0.011, p=0.008 respectively), indicative of reduced endothelial function with higher maternal third trimester serum ferritin, though multiple regression models were inconclusive. This is a novel and potentially important clinical finding that merits further study.

5.1.2. Future Directions

Our findings should prompt further exploration of the impact of GDM on the myocardial and vascular health of the child. In addition to strain analyses, other measures of CVD risk, including a family history, and further exploration of the metabolic status of the child including insulin resistance, lipid profile, and evidence of inflammation, would provide a more complete picture of the impact of this prevalent adverse exposure. Our study included only a small number of males and, as such, did not permit exploration of the role of biological sex, a factor known to modulate the effect of prenatal insults, including maternal diabetes [88]. Future studies with more

male participants are needed to explore sex-related differences in CV outcomes following exposure to maternal GDM.

While CV alterations following exposure to maternal diabetes have been observed in human and animal models as described in Chapter 1, there is a paucity of studies that investigate responsible mechanisms and the evolution of disease. The presence of increased aortic stiffness in children of mothers with diabetes could reflect changes in the extracellular matrix, including altered relative amounts of elastin and collagen and/or organization of the vascular walls [59,307,312–316]. What drives these changes is unknown. Our finding of greater aortic stiffness in children of mothers on insulin could suggest worse glycemic control and/or insulin itself may be important contributors. Possible instigators that should be explored include inflammation and oxidative stress [361,363], as well as epigenetic alterations [370] leading to changes in gene expression that could negatively impact aortic development at organ, cellular, and molecular levels.

This area of research is paramount to understanding responsible mechanisms and defining effective strategies to reduce long-term CVD risks in children born to mothers with GDM. There may be targeted antioxidants or other medical interventions applied pre- and postnatally that could mitigate the negative consequences of GDM on the developing CV system of the fetus. Further, as insulin was the sole medication used for GDM management in our study, future investigations should examine the possible impact of using alternative medications such as metformin or glyburide. While insulin is recommended in Canada, metformin and glyburide are recognized as acceptable alternatives in pregnancy [371]. Beyond medication, it would also be of merit to investigate the impact of earlier screening and management, as well as the positive impact of earlier dietary approaches to manage or even possibly prevent GDM.

While vascular changes have been detected following exposure to pregestational diabetes from infancy to adolescence [122,133,261], there have been fewer studies investigating such changes following exposure to GDM. One study investigated fetal vascular alterations in pregnancies complicated by diabetes, including both gestational and pregestational, but the effect of GDM was not analyzed separately [265]. The Finnish Gestational Diabetes Prevention (RADIEL) study found in six-year-old children of mothers at high risk of developing GDM that first and second trimester fasting glucose predicted carotid arterial stiffness [145]. Future work should investigate fetal vascular changes in pregnancies complicated by GDM specifically as well as whether vascular alterations persist beyond childhood and into adolescence and early adulthood. The MySweetHeart Cohort study represents one such study that is currently underway [372].

5.2. Surgical Outcomes in Infants with Major Congenital Heart Disease Exposed to Maternal Diabetes in Utero

5.2.1. Summary of Findings

In our secondary study, we found a suggestion of worse outcomes in infants undergoing cardiopulmonary bypass (CPB) surgery for congenital heart disease (CHD) following intrauterine exposure to pregestational diabetes. Comparing infants (IDM) of mothers with pregestational diabetes and controls in the lower risk adjustment for congenital heart surgery (RACHS) [356] Group A, we found IDM to have longer hospital stays (19.5±29.3 vs 9.8±6.5 days, p=0.046) and higher urea levels (11.0±4.4 vs 8.4±4.2 mmol/L, p=0.035). Despite small patient numbers, we also found trends towards worse outcomes in the higher risk subgroup RACHS Group B with higher pediatric risk of mortality (PRISM) score [359] (15.6±8.8 vs 10.0±5.3, p=0.11), and higher postoperative serum glucose (16.7±1.3 vs 14.3±2.7 mmol/L, p=0.059).

When considering outcomes in the pooled diabetes analyses however, including gestational and pregestational diabetes, versus controls, we did not find statistically significant differences in any outcomes. This finding was likely driven by the larger proportion of children exposed to maternal GDM included in the study who had on whole similar or better outcome measures than controls. IDM with GDM mothers in RACHS Group A demonstrated shorter intensive care stay (3.0±2.6 vs 3.8±3.1 days, p=0.022), and those in RACHS Group B demonstrated lower postoperative glucose (13.4±2.0 vs 16.7±3.0 mmol/L, p=0.014) than their control counterparts. These findings could provide evidence of a differential effect of the type of maternal diabetes on susceptibility to ischemia-reperfusion injury (IRI) and/or other insults related to these exposures.

5.2.2. Future Directions

Taken together, our findings, and the compelling observations in animal models that suggest greater susceptibility to IRI in adulthood following fetal exposure to maternal diabetes [124,174], should prompt and will inform future larger multicenter studies designed to explore the impact of maternal diabetes exposure to CHD outcomes. Further studies should include exploration of changes in myocardial function through use of echocardiography as well as inclusion of better measures of cardiac output and inotrope use. The relative rarity of the patients with major CHD requiring surgical repair or palliation through use of CPB in infancy who had also been exposed to maternal diabetes before birth dictates the need for a multicenter center study to explore more subtle findings particularly in the face of variability in cardiac pathology among many other confounding factors in this patient population [373]. With a larger multicenter study, it would be important to stratify infants by exposure to the different type of diabetes and to include important confounders in the models such as mode of birth, gestational age at birth and

birth weight, maternal age, maternal hemoglobin A1c throughout pregnancy, and demographic variables. Further subcategorization of CHD and inclusion of factors such as length of CPB and circulatory arrest in the model would also be prudent given the potential contribution of such factors to the degree of IRI.

Definition of the cardiac insults associated with an exposure to maternal diabetes is important as this could lead to the evolution of innovative preoperative, operative, and postoperative strategies to prevent or reduce injury. In fact, a rat model of maternal diabetes previously identified maternal treatment with melatonin, a potent antioxidant [374], to improve outcomes in adult offspring following myocardial ischemia and reperfusion [174]. This could provide evidence not yet explored in humans that prenatal interventions have the potential to improve postnatal outcomes associated with maternal diabetes and that oxidative pathways may be key contributary mechanisms, providing an impetus for further study.

5.3. Conclusions

Diabetes in pregnancy has proven to be an important risk factor in the overarching concept of CV programming. This thesis provides additional evidence of its importance and helps establish the groundwork for future studies in the area, including investigation of the differential impacts of the different types of diabetes. Though insulin imbalance, hyperglycemia, inflammation, and oxidative stress are common among gestational, type 1, and type 2 diabetes, there are important differences among these conditions, including timing of onset (with respect to stages of CV development, susceptibility, and length of exposure), need for exogenous insulin therapy, and other factors such as adiposity and genetics, that may have implications for the offspring's CV health. Our work investigating the impact of GDM on the CV health of the child

has provided strong evidence of early vascular changes in exposed children, while our investigation of surgical outcomes in infants with CHD suggested divergent outcomes for infants exposed to gestational (better) versus pregestational (worse) diabetes relative to infants without such prenatal exposures. These two very different studies add to our understanding of fetal CV programming in pregnancies complicated by diabetes and illuminate important directions for future research.

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