THE ASSOCIATION BETWEEN GESTATIONAL DIABETES MELLITUS AND
CARDIOVASCULAR DISEASE AND POSSIBLE MEDIATION BY TYPE 2 DIABETES
MELLITUS

by

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This thesis is dedicated to my late father, Jack Carew, who passed away in 2014 at the age of 58 from complications arising from type 2 diabetes. His unwavering love, support, and encouragement throughout my upbringing has made completing this thesis possible.

My Dad and me.

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ABSTRACT

This retrospective cohort study quantified the risk of subsequent cardiovascular disease (CVD) associated with gestational diabetes mellitus (GDM) and estimated the extent to which type 2 diabetes mellitus (T2DM) mediates this association. Cox regression models were used to quantify the association between GDM and CVD, while mediation analysis was used to determine whether T2DM mediates this association. Analyses used data from a linkage of the Nova Scotia Atlee Perinatal Database with administrative health databases.

Among a total of 87,632 women who were included, 4.0% experienced GDM in at least one pregnancy. Women were followed for a median of 11.8 years and 1.4% developed CVD. Compared with women who did not have GDM, women with GDM had a higher risk of developing CVD [adjusted HR=1.53 (95% CI 1.23-1.91)]. T2DM mediated 93.9% of this association. These results support screening and counseling women with prior GDM to improve cardiovascular health.

LIST OF ABBREVIATIONS USED

aHR	Adjusted Hazard Ratio
aOR	Adjusted Odds Ratio
BMI	Body Mass Index
ccIMT	Common Carotid Intima-Media Thickness
CHD	Coronary Heart Disease
CI	Confidence Interval
CVD	Cardiovascular Disease
DAD	Discharge Abstract Database
GCT	Glucose Challenge Test
GDM	Gestational Diabetes Mellitus
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
HDL	High Density Lipoprotein
HDNS	Health Data Nova Scotia
HR	Hazard Ratio
ICAM-1	Intercellular Adhesion Molecule-1
ICD	International Classification of Diseases
IHD	Ischaemic Heart Disease
LDL	Low-density lipoprotein
MI	Myocardial Infarction
NSAPD	Nova Scotia Atlee Perinatal Database
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PAD	Peripheral Artery Disease
PCOS	Polycystic Ovary Syndrome
PG	Plasma Glucose
QAIPPE	Quintile of Annual Income Per Person Equivalent
RCP	Reproductive Care Program
RR	Relative Risk

SES	Socioeconomic Status
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TIA	Transient Ischaemic Attack
uHR	Unadjusted Hazard Ratio
VCAM-1	Vascular Cellular Adhesion Molecule-1

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Chapter 1 INTRODUCTION

Cardiovascular disease (CVD), including heart disease and stroke, continues to be a major health challenge for women. Cardiovascular disease surpassed cancer as the leading cause of death for women in Canada, contributing to 31,294 deaths in 2013 (1). Cardiovascular disease is also a major economic burden on the Canadian health care system both directly (e.g., more frequent physician visits and increased hospital admissions) and indirectly (e.g., loss of economic contributions due to disability and premature mortality). Cardiovascular disease was once thought to be a "disease of aging" as pre-menopausal women tend to be safeguarded by the protective effects of estrogen; however, young women are now experiencing multiple CVD risk factors such as obesity, physical inactivity, type 2 diabetes (T2DM), hypertension, and cigarette smoking early in their lives, and this is expected to translate into a dramatic increase in the number of women with the disease in the future (2). For example, approximately 1.7 million Canadian women aged 20 to 34 are inactive and nearly one million are overweight (2). It is important to identify mechanisms that contribute to the disease to identify women at high risk and intervene at the earliest possible time point (2).

Women may develop certain conditions during pregnancy such as gestational diabetes mellitus (GDM), which put them higher risk of developing CVD (3). Between three and twenty percent of pregnant women worldwide develop GDM, depending on their risk factors and the criteria used for diagnosing GDM, and studies show that the prevalence of GDM may be increasing over time (4). Gestational diabetes mellitus is described as hyperglycemia during pregnancy, or glucose intolerance, which usually resolves postpartum. Mild insulin resistance normally occurs during pregnancy as a mechanism to provide sufficient blood glucose for the developing fetus and a woman's pancreatic β-cells usually respond to this insulin resistance by

naturally increasing insulin secretion. In GDM, however, a defect in these pancreatic β-cells leads to an insufficient insulin supply to compensate for this insulin resistance, resulting in the mother's metabolic demands not being met (5).

Existing literature has demonstrated that insulin resistance and glucose intolerance are associated with elevated CVD risk later in life and, therefore, women who develop GDM are at higher risk of developing this disease in the years following pregnancy (6). Women with a history of GDM also experience CVD events at a younger age compared to women without a history of GDM (7). Women who develop GDM are also at higher risk of overt T2DM later in life, independent of common risk factors [RR=7.4 (95% CI: 4.8-11.5)] (8). A diagnosis of T2DM has also been shown to raise the risk of developing CVD (9,10). It is still unclear whether the association between GDM and CVD is mediated by the increased risk of CVD associated with T2DM as there have been conflicting results reported in the literature (7,11,12). Limitations in the methods used for examining mediation and lack of adjustment for all potential confounders may have contributed to the lack of consistent findings.

The aim of this population-based retrospective cohort study was to determine whether women with GDM have an increased risk of developing CVD following pregnancy and whether this association is mediated by T2DM. This aim was achieved by using up to 26 years of follow-up data on nearly 88,000 Nova Scotian women. This study took advantage of comprehensive information about pregnancy conditions and maternal characteristics recorded in the Nova Scotia Atlee Perinatal Database (NSAPD). These data were linked to administrative health service utilization data to identify the occurrence of T2DM and CVD. This study examined the relationships between GDM, T2DM, and CVD by conducting a formal mediation analysis and appropriately adjusted for potential confounders in statistical models. As a result, it addressed

important limitations in the existing literature and provided an estimate of the direct effect of GDM (that which is not mediated by the intermediate development of T2DM) on the risk of developing subsequent CVD. The results of this study have the potential to contribute to the scientific literature and help guide public health policy and clinical practice guidelines with respect to early CVD risk factor assessment and modification.

Chapter 2 BACKGROUND AND LITERATURE REVIEW

The associations between GDM and T2DM, and T2DM and CVD have been well-established in the literature in recent years (8-10); however, the extent to which GDM is associated with CVD and whether this risk is independent of T2DM remains unclear (7,11,12). This literature review will start with definitions of both GDM and CVD and descriptions of the pathophysiology and epidemiology of these conditions. An overview of the risk factors associated with these conditions will then be discussed, which will demonstrate the risk factors that should be considered as potential confounders in regression models used to address the objectives of this study. Epidemiologic evidence for the associations of direct relevance to this study, those between GDM, T2DM, and CVD, will then be reviewed in depth.

2.1 Gestational Diabetes Mellitus

2.1.1 Definition of Gestational Diabetes Mellitus

Women normally enter a mild insulin resistant state during pregnancy that begins near mid-pregnancy and continues into the third trimester (13). This target cell resistance to insulin is likely a physiological mechanism to ensure adequate blood glucose for the developing fetus as glucose homeostasis is restored to pre-pregnancy levels shortly after delivery (14). In GDM, however, this level of insulin resistance is exaggerated and is comparable to that of patients with T2DM (15). Gestational diabetes mellitus may be attributed to a combination of increased maternal adiposity and placental hormones such as estrogen, progesterone, human placental lactogen, human placental growth hormone, and cortisol that antagonize the action of insulin (16). Unlike T2DM, GDM is considered a transient condition as glucose intolerance often

resolves to pre-pregnancy levels in the immediate postpartum period (13), although women with GDM are more likely to develop long-term health issues such as overt T2DM in the future (8).

Pathophysiology

Pregnancy provides a physiological stress test of the body's ability to regulate normal glucose levels and can, therefore, unmask metabolic defects and maternal vulnerability to future chronic metabolic abnormalities (17). A woman's pancreatic β-cells aid in regulating normal glucose levels by naturally increasing their insulin secretion in response to pregnancy-induced insulin resistance, thereby demonstrating a high degree of plasticity in these cells (13). This results in a minimal fluctuation in circulating blood glucose levels despite large changes in insulin sensitivity. In GDM, a β-cell dysfunction arises where the supply of insulin produced by these cells is insufficient to compensate for the insulin resistance and, therefore, the mother's metabolic demands are not met. It was generally thought that GDM arose in women whose β-cells were not able to increase insulin production to meet the insulin supply demands during pregnancy; it is now believed that GDM manifestation represents chronic β-cell dysfunction (18,19). Previous studies have shown that a reciprocal relationship between insulin sensitivity and insulin secretion exists in both women with GDM and without GDM (19). This observation indicates that β-cells compensate for insulin resistance in women with GDM, although the amount of insulin secreted for each degree of insulin sensitivity is lower for women with GDM (19). This compensation is reduced to a similar degree postpartum in women with GDM, indicating that the defect in β-cell function is chronic and is not acquired during pregnancy (20,21). As a result, women with GDM are at substantially higher risk of developing T2DM later in life than their non-GDM counterparts.

Screening for Gestational Diabetes Mellitus

Prior to 2013, the Canadian Diabetes Association (which is now Diabetes Canada) recommended diagnosing GDM using a two-step approach that involved a series of laboratory blood tests: the glucose challenge test (GCT), which is a screening test, and the oral glucose tolerance test (OGTT), which is a diagnostic test (22). All women at 24-28 weeks' gestation would be initially screened with a 1-hour 50-g GCT. A value of >10.3 mmol/L indicated a positive GDM diagnosis. Values that fell between 7.8 mmol/L and 10.3 mmol/L indicated that an OGTT should be performed. A woman was diagnosed with GDM if at least two of three values were abnormal (fasting glucose ≥ 5.3; 1-hour plasma glucose (PG) ≥ 10.6; 2-hr PG ≥ 8.9 mmol/L). A diagnosis of impaired glucose tolerance was given if one of these values was abnormal.

Diabetes Canada revised their GDM screening guidelines in 2013 to reflect new evidence on diagnostic criteria from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study (23). The objective of this study was to estimate the risk of adverse perinatal outcomes associated with maternal glucose intolerance that did not meet the criteria for GDM at the time (24). The HAPO study group concluded that a strong, continuous association exists between maternal glucose levels below the cutoff for a GDM diagnosis and increased birth weight and increased cord-blood serum C-peptide levels (24). These findings prompted changes to screening guidelines and diagnostic criteria among several international diabetes organizations including the Canadian Diabetes Association, but consensus on a common approach has not been reached (25). The International Diabetes Federation currently oversees 230 national diabetes associations in over 170 countries and territories. Many of these national associations offer their own

guidelines on approaches to screening and diagnosing GDM, but they are often in disagreement with regional diabetes and obstetrical organizations' recommendations.

The Diabetes Care Program of Nova Scotia adopted GDM screening guidelines from the 2013 version of Diabetes Canada's Clinical Practice Guidelines and currently recommends a two-step approach for women in Nova Scotia (26). This screening approach involves screening all pregnant women at high risk for GDM (i.e., previous diagnosis of GDM, prediabetes, history of a macrosomic infant, member of a high-risk population (Aboriginal, Hispanic, South Asian, Asian, African), age $\geq$ 35 years, body mass index (BMI) $\geq$ 30 kg/m^2, polycystic ovary syndrome (PCOS), acanthosis nigricans, corticosteroid use, multiple gestation, history of unexplained stillbirth, 1st degree relative with T2DM, glycosuria, pancreatic insufficient cystic fibrosis, current fetal macrosomia or polyhydramnios) as early as possible in the pregnancy, preferably in the first trimester, with a random 50-g GCT and 1-hour PG (26). A woman is diagnosed with GDM at this time if she has a value $\geq$ 11.1 mmol/L on the 50-g GCT (26). All women not previously found to have overt diabetes or GDM during earlier screening in the pregnancy are then screened with a 50-g GCT at 24-28 weeks' gestation followed by a 2-hour 75-g OGTT if the result of the GCT fell between 7.8 mmol/L and 11.0 mmol/L (26). Gestational diabetes mellitus is diagnosed if one or more of the following values exceeds the cutoff: fasting PG $\geq$ 5.3; 1-hour PG $\geq$10.6; 2-hour PG $\geq$ 9.0 mmol/L (26). However, a 75-g OGTT can be performed using the same cutoffs without an initial GCT if a woman has prediabetes, prior GDM, or 3 or more risk factors (i.e., GDM is strongly suspected) (26).

2.1.2 Descriptive Epidemiology of Gestational Diabetes Mellitus

Heterogeneity in screening approaches and diagnostic criteria based on blood glucose threshold values that must be met or exceeded to define GDM across international diabetes organizations such as the American Diabetes Association (27), World Health Organization (28), National Institute for Health and Care Excellence (29), and Diabetes Canada (23) make it difficult to estimate the prevalence of GDM. The inherent characteristics of study populations and diagnostic criteria used often differ between studies, making prevalence estimates difficult to compare. Particularly, recent studies are more likely to estimate or re-evaluate GDM prevalence using the new International Association of the Diabetes and Pregnancy Study Groups criterion, which tend to estimate a higher prevalence of GDM compared to other criteria used in the past (30). These disparate estimates warrant caution when interpreting results.

A recent study by Zhu et al. (31) attempted to comprehensively synthesize data published in the past decade to estimate the global prevalence of GDM by country and region. The authors reported that the Middle East and North Africa had the highest rates of GDM with a median estimate of 12.9% (range: 8.4%-24.5%). Europe had a median estimate of 5.8% (range: 1.8%-22.3%), making it the region with the lowest prevalence of GDM compared to the other regions included in the study. In North America and the Caribbean, the estimated median prevalence fell in between these estimates (7.0%). The prevalence of GDM in Canada was 6.5% based on the Canadian studies included in the analysis. These results, however, should be interpreted with caution as the variability in regional estimates may be partly attributable to differences in diagnostic criteria that were applied.

A recent meta-analysis of 40 studies that collected data in Europe supported results found in the Zhu et al. review (32). The investigators found that the overall mean prevalence in Europe

was 5.4% (95% CI: 3.8%-7.8%) and that the prevalence of GDM did not differ significantly between studies using one-step and two-step screening. When stratified by region, Southern Europe had the highest prevalence at 9.6% (95% CI: 7.3%-12.6%) whereas Northern Europe had the lowest prevalence at 2.3% (95% CI: 1.3%-3.8%) (32).

The Public Health Agency of Canada reported that the rate of GDM ascertained from the Canadian Institute for Health Information's Discharge Abstract Database (DAD) (based on all provinces except Québec) between 2010 and 2011 peaked at 5.45% (95% CI: 5.36%–5.54%), steadily increasing from 4.08% between 2004 and 2005 (33).

Gestational diabetes mellitus is becoming a growing concern for society as the prevalence continues to rise over time, with greater increases observed in ethnic and racial minority groups. A study that included the entire population of the United States (58,922,266 births between 1989 and 2004) found that the prevalence of GDM showed a relative increase of 122% between 1989 and 2004 and that this trend was consistent across all geographic areas of the country (34). A black-white disparity in this trend was found as the prevalence of GDM increased by 80% in white women and by 172% in black women (34). This temporal increase in prevalence is large but may be partly attributable to increased screening coverage and the revision of PG criteria for the diagnosis of GDM (34).

A recent population-based cohort study in Ontario, Canada found a doubling of the incidence of both GDM and pre-GDM between 1996 and 2010, after adjusting for age (35). This incidence rose in parallel to increasing rates of obesity, decreased physical activity, increased saturated fat content in diets, and T2DM, leading some to believe there may be a connection between these factors (36). These results were consistent with another study that reported that the average prevalence of GDM in Manitoba, Canada was significantly positively correlated with

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time with a rise in prevalence from 2.3% to 3.7% between 1985 to 2004 (37). This study, in addition to another in Manitoba, found that GDM disparities exist between First Nations women and non-First Nations women as they revealed that First Nations women had two to three times more GDM than non-First Nations women (37,38).

The incidence of GDM also continues to increase in Nova Scotia. The most recent (2014) estimated cumulative incidence of GDM in Nova Scotia is approximately 6.4% of pregnancies, up from 2.6% in 2001 (39). This estimate is based on data ascertained from the NSAPD.

2.1.3 Established Risk Factors for Gestational Diabetes Mellitus

Several risk factors for the development of GDM have been established including obesity, advanced maternal age, and family history of diabetes. It is imperative to identify women who are at high risk of developing GDM early in pregnancy given the rising rate of GDM in recent years. Ideally, women who are identified as being at high-risk can be presented with intervention options early in an effort to decrease the risk of GDM and its associated adverse outcomes, including T2DM and CVD (40).

A shift towards more sedentary lifestyles and diets that are high in sugar and fat have contributed to the current global pandemic of obesity, which is usually defined as having a BMI of 30 kg/m^2 or higher (41,42). Body mass index, a value derived by dividing a person's weight (in kilograms (kg)) by the square of their height (in metres), is a commonly used anthropometric measure and is associated with GDM. Focus on the association between maternal obesity and GDM has become a priority research area in recent years in hopes of breaking the vicious cycle involving maternal GDM, childhood and maternal obesity, and T2DM (43). Torloni et al. (44) estimated unadjusted odds ratios (OR) in their meta-analysis of 3.76 (95% CI: 3.31-4.28), 3.01

(95% CI: 2.34-3.87), and 5.55 (95% CI: 4.27-7.21) respectively, for women with obesity (BMI $\geq$ 30 kg/m^2), moderate obesity (BMI 30-34.9 kg/m^2), and morbid obesity (BMI $\geq$ 35 kg/m^2), when compared with women of normal weight. Other cohort studies from various countries have found a similar risk of developing GDM associated with obesity since this meta-analysis was published (45,46). Maternal obesity is an important risk factor because of its high prevalence and strong association with GDM.

Childbearing over the age of 35, which is the age that is typically used to mark advanced maternal age, was found to be associated with GDM (47,48). A recent study involving over 125 million pregnancies in the United States found that the relative risk (RR) of GDM increased with age (47). The unadjusted RR of GDM in the 15-19 age group was 0.35 (95% CI: 0.35–0.35) and increased to 3.23 (95% CI: 3.16–3.29) in the 45-49 age group compared to the reference group of 25-29-year-old women (47). Gestational diabetes mellitus appears to increase linearly across maternal age (48).

More women today are entering pregnancy overweight or obese and are gaining too much weight according to the Institute of Medicine's BMI-specific recommendations for gestational weight gain (49). Gestational weight gain greater than these recommendations is associated with an increased risk of GDM. MacDonald et al. (50) found that there was a 24% (95% CI: 9%-53%) increased odds of GDM with every standard deviation increase in total weight gain at screening for GDM among women of normal weight, after adjusting for potential confounders. This finding is in keeping with a recent meta-analysis that included eight studies and 13,748 participants that estimated an unadjusted summary OR of 1.40 (95% CI: 1.21-1.61) (51). This OR increased to 1.42 (95% CI: 1.20-1.68) after conducting a sensitivity analysis that

included only studies that adjusted for confounders including maternal age, ethnicity, smoking, pre-gravid BMI, blood pressure at the first prenatal visit, parity and history of GDM (51).

Rates of hypertension are on the rise in Canada among young women and this is concerning as hypertension has been identified as a potential risk factor for GDM (2,52). A nested case-control study found that women with hypertension in early pregnancy had a twofold increase in risk of developing GDM compared to women who were normotensive, after adjustment for age, race/ethnicity, gestational week of blood pressure, BMI, and parity [adjusted odds ratio (aOR)=2.04 (95% CI: 1.14–3.65)] (52). Women who had prehypertension, which is defined as systolic blood pressure of 120–139 mmHg and/or diastolic blood pressure of 80–89 mmHg, early in pregnancy also had an elevated risk of developing GDM compared to women who were normotensive [aOR=1.56 (95% CI: 1.16–2.10)] (52). The odds of developing GDM was slightly lower among women with prehypertension compared to women with hypertension, albeit it was still significant and positive and, therefore, this indicates that the risk of developing GDM may increase with increasing blood pressure levels.

Polycystic ovary syndrome is a relatively common endocrine disorder affecting approximately 6-19% of women of reproductive age (53). Polycystic ovary syndrome in non-pregnant women is associated with an irregular menstrual cycle, decreased fertility, insulin resistance, obesity, hyperandrogenism, and diabetes mellitus- both T2DM and GDM (54). The insulin resistance associated with PCOS has an additive effect on the natural baseline insulin resistance level in pregnancy and, therefore, may worsen or lead to GDM (55). A meta-analysis of pregnancy outcomes associated with PCOS yielded an OR for GDM of 2.82 (95% CI: 1.93-4.10) (55). Results from a more recent prospective cohort study estimated an OR of 3.25 (95% CI: 1.54-6.88) after adjustment for age, parity, and BMI (56).

As previously mentioned, pregnancy induces insulin resistance that begins near mid-pregnancy and continues through the third trimester as a physiological mechanism to ensure adequate blood glucose for the developing fetus (13). The pancreatic β-cells usually compensate for this insulin resistance by increasing the secretion of insulin. Having more pregnancies and, therefore, multiple episodes of insulin resistance, may result in a decline of β-cell function and increase the risk of GDM in later pregnancies (57). Epidemiologic evidence supports this hypothesis (58). A systematic review and meta-analysis identified four studies that examined the association between parity and GDM (58). Three of these studies found that the odds of GDM were elevated among multiparous women and that the odds of GDM recurrence were between 3.5 and 4.0 times higher in multiparous women compared to primiparous women (59-61).

Race and ethnicity also appear to be associated with the development of GDM. A historic, but important, study conducted by Berkowitz et al. reported that Native American, Asian, Hispanic, and African-American women have an increased risk of GDM compared to non-Hispanic white women (62). Similarly, women of Asian or South Asian ethnicity have increased insulin resistance, a characteristic of GDM, in late pregnancy compared to women of Caucasian heritage, independent of age, weeks' gestation, parity, pre-pregnancy BMI, weight gain in pregnancy, glucose intolerance, previous history of GDM, and family history of T2DM (63). Ethnicity modifies the association between pre-pregnancy BMI and insulin resistance as pre-pregnancy BMI has a greater effect on Asian women compared to Caucasian women and has a relatively modest effect on South Asian women (63). As noted above in section 2.1.2, First Nations women, the most populous Indigenous group of women in Canada, experience a disproportionate burden of GDM. The overall rates of GDM in Saskatoon were estimated to be approximately 3.5% for non-Indigenous women and 11.5% for Indigenous women (64).

Multivariable analysis demonstrated that First Nations ethnicity was an independent predictor of GDM; however, the effect was most notable when combined with obesity (64).

Anna et al. (65) found that socioeconomic status (SES) is inversely associated with the risk of developing GDM. Women living in the lowest SES quartile in New South Wales, Australia had a 65% (95% CI: 60%-70%) higher risk of GDM compared to women living in the highest SES quartile of this region and this association was consistent across various ethnic groups. A Canadian study conducted in Toronto found that women living in areas that are materially deprived and that have high concentrations of ethnic minorities tended to have higher glucose levels at diagnosis of GDM and hence, may have had more severe GDM (66).

A family history of diabetes has been identified as a risk factor for GDM in several studies although a maternal predominance of T2DM in the family history of women with GDM may exist (67-69). Harder et al. (70) found that the prevalence of T2DM was significantly greater in mothers than in fathers of women in their study with GDM.

2.2 Cardiovascular Disease
2.2.1 Definition of Cardiovascular Disease

Cardiovascular disease is an umbrella term describing a class of non-communicable diseases affecting the heart and blood vessels resulting from interactions between genetic and environmental factors, including health behaviours (71,72). Cardiovascular disease is usually associated with a build-up of fatty deposits on the walls of the arteries - a process termed atherosclerosis - and is less often associated with bacterial infections. The build-up of these fatty deposits or plaque causes the arteries to become irregular and the lumen of the artery to narrow which, in turn, restricts blood flow. If a blood clot forms, this narrowing can potentially be

detrimental or fatal as blood flow can cease and heart attack or stroke can result (73,74). The four main types of CVD are ischaemic heart disease (IHD) including angina, heart attacks, and heart failure; cerebrovascular disease including strokes and transient ischaemic attacks (TIA); peripheral arterial disease (PAD); and aortic disease including aortic aneurysm and dissection. Another type of CVD, although less common in developed countries such as Canada, is rheumatic heart disease, which is caused by bacterial infections attacking the body's joints and heart valves (75).

Pathophysiology

Atherosclerosis, a chronic inflammatory disorder affecting medium- and large-sized blood vessels, is the main pathological process driving IHD and cerebrovascular disease. This process is described in detail in an article by James Scott (76) and has been briefly summarized here. Atherosclerosis occurs because of endothelial dysfunction caused by irritants such as low-density lipoprotein (LDL) cholesterol and free radicals. A breach in the endothelium ensues, making it permeable to lymphocytes and monocytes. These cells then move into deeper layers of the blood vessels where a series of reactions occur that attract LDL to the site. LDL particles are then engulfed by monocytes, which convert into macrophages and foam cells because of this engulfment. Smooth muscle cells then migrate from the tunica media layer of the blood vessel to the tunica intima, the luminal layer, and produce a fibrous cap consisting of collagen and elastin. Simultaneously, the macrophages involved in the initial reaction die and contribute to the necrotic core that is covered by the fibrous cap. The smooth muscle cells then start calcifying this fibrous cap, which contributes to arterial stiffness and decreased compliance and flexibility. Meanwhile, cells and lipids accumulate in the plaque structure, which continues to bulge into the lumen of the blood vessel. This plaque structure poses a danger as the fibrous cap can thin and

the epithelial surface of the plaque can become fissured, eventually breaking away from the vessel wall where cellular debris and lipid fragments become released into the vessel debris. A coronary blood vessel or cerebral blood vessel can become blocked if thrombogenic agents attach to this material and form a blood clot, resulting in a heart attack or stroke, and potentially death.

2.2.2 Descriptive Epidemiology of Cardiovascular Disease

The prevalence and incidence of CVD, which is the leading cause of admission to hospital, disability, and premature death worldwide for both men and women, remains high (77,78). The World Health Organization estimated in a 2011 report that CVD accounted for over 17.3 million deaths globally in 2008, corresponding to approximately 30% of all deaths (79). They also estimated that the number of CVD deaths is expected to climb to 23.6 million by 2030 (79). Cardiovascular disease also severely burdens the health of affected individuals. The World Health Organization estimated that CVD is responsible for 151,377 million lost years of healthy life worldwide, a metric known as disability-adjusted life years (79). In Canada, 6% of Canadians 20 years and older reported that they lived with CVD in 2014, although the proportion of Canadians reporting that they were living with CVD increased with age (80). The proportion of Canadians age 65 years and older reporting that they lived with a CVD in 2014 was approximately 18% (80). The age-standardized prevalence of Canadians with CVD has remained stable since 2007 (80). This equates to approximately 2.4 million adult Canadians with heart disease and another 741,800 living with stroke. Rates of death attributable to CVD have declined over time, mainly due to greater focus on prevention, risk factor reduction, more effective diagnoses and treatment, and better disease management (81).

Statistics Canada reported that in 2013, 23,437 Canadian women died of heart disease and 7,857 women died of stroke (1). This comprises 18.7% and 6.3% of the total causes of death, respectively (1). Heart disease comprised a greater percentage of the total causes of death for men at 20.8%; however, stroke could be attributed to 4.4% of deaths in 2013 in men, fewer than what was seen in women (1). Overall, there is a downward trend in rates of death from CVD in women in Canada. Provincial differences in prevalence, incidence, death rates, and associated risk factors exist and demonstrate an East-West gradient. Newfoundland and Labrador tends to fare worst with regards to these rates whereas British Columbia has the lowest rates in the country (82). In Nova Scotia, diseases of the heart were the second leading cause of death among women, while cerebrovascular diseases were the third leading cause of death among women (82). Rates of death from diseases of the heart in 2008 were 162.7 per 100,000 women, down from 196.1 per 100,000 women in 2003 (82). The rate of death from cerebrovascular disease was 70.6 per 100,000 women in 2008, up from 67.4 per 100,000 women in 2003 (82).

2.2.3 Established Risk Factors for Cardiovascular Disease

Cardiovascular disease manifests differently in men and women likely due to sex differences in major risk factors and biological differences in atherosclerosis (83). Historically, CVD, particularly heart disease, was misperceived as a "man's disease" and a "disease of aging" despite being one of the leading causes of morbidity and mortality in women (84). Studies show that pre-menopausal women may be protected from CVD by estrogen and that these women have an advantage over men as they develop heart disease, on average, nine years later than men (85); however, the assumption that the risk of CVD in pre-menopause is not high has resulted in under-recognition of the disease, less aggressive cardiovascular treatment strategies for women, and guidelines regarding diagnostic and therapeutic procedures being overlooked by physicians

(86). In fact, women today are experiencing multiple CVD risk factors such as T2DM, obesity, smoking, physical inactivity, and hypertension in early and midlife, which is likely contributing to earlier onset of CVD (2).

Cigarette smoking increases the risk of atherosclerosis in coronary, cerebral, and peripheral arteries and is associated with more severe coronary lesions (87,88). A meta-analysis demonstrated that the association between cigarette smoking and IHD follows a dose-response relationship where smoking 1-19 cigarettes per day and 20 or more cigarettes per day led to respective RRs of 1.23 (95% CI: 1.13-1.34) and 1.31 (95% CI: 1.21-1.42) in comparison to non-smokers (89). Smoking is a key risk factor for CVD in both men and women; however, its effect is exacerbated in women (90). A meta-analysis of 26 studies that included 3,912,809 individuals and 67,075 coronary heart disease (CHD) events found that women who smoked had a 25% (95% CI: 12%-39%) greater risk of experiencing CHD relative to men who smoked independent of other cardiovascular risk factors and after adjustment for potential publication bias (90). Based on data published by Statistics Canada in 2016 a greater percentage of women smoke in Nova Scotia compared to the national average (18.1% vs. 16.9) (91).

Increased BMI and excessive adipose tissue accumulation are associated with subclinical metabolic and vascular dysfunction, along with CVD risk and excess mortality risk. Kramer et al. (92) compared metabolically healthy obese (BMI $\geq$ 30 kg/m^2) and normal weight (BMI 18.5-25 kg/m^2) individuals in their meta-analysis and found that the obese individuals had a 24% (95% CI: 2%-55%) increased risk of experiencing all-cause mortality or cardiovascular events after a median of 10 years of follow-up. Interestingly, all metabolically unhealthy individuals regardless of their BMI were at increased risk of CVD and mortality when compared to metabolically healthy normal weight individuals [Normal weight RR=3.14 (95% CI: 2.36-3.93),

Overweight RR=2.70 (95% CI: 2.08-3.30), Obese RR=2.65 (95% CI: 2.18-3.12)] and, therefore, both BMI and metabolic health should be accounted for when assessing future CVD risk (92).

Energy imbalance through physical inactivity and unhealthy diet is also related to CVD risk. In Canada, 78.3% of women aged 18-39, 84.9% of women aged 40-59, and 87.1% of women aged 60-79 reported physical activity levels that did not meet the Canadian guidelines in 2015, which recommend accumulating at least 150 minutes of moderate-to-vigorous physical activity per week (93). Physical inactivity has been shown to have an impact on CVD comparable to that of smoking (94). Chomistek et al. (95) found that sitting $\geq$ 10 hours per day compared to $\leq$ 5 hours per day was associated with 18% greater CVD risk (95% CI: 9%-29%) and that this association was strongest in overweight versus normal weight women and women 70 years of age or older compared with younger women. Correspondingly, recent meta-analyses found that a high level of leisure time physical activity reduces the risk of CVD in the range of about 20-30% compared to a low level, while a moderate level reduces the risk by approximately 10-20%, thereby illustrating a dose-response relationship (96,97).

Additionally, diets high in fat, sugar, and salt have been shown to increase the risk of CVD. Higher salt intake was associated with 23% (95% CI: 6%-43%) greater risk of stroke and 14% (95% CI: 1%-32%) greater risk of total CVD in a meta-analysis of prospective studies (98). Similarly, a systematic review of randomized controlled trials involving approximately 53,300 participants in 13 studies found that reducing saturated fat by reducing overall fat intake reduced the risk of cardiovascular events including non-fatal myocardial infarction (MI), angina, stroke, heart failure, peripheral vascular events, and atrial fibrillation by 17% (95% CI: 4%-28%) (99). High sugar intake was also found to be associated with increased CVD risk. Yang et al. (100) found that participants in their study who consumed 10.0-24.9% of calories from added sugar

had 1.30 (95% CI: 1.09-1.55) times greater risk of CVD than those who consumed less than 10%

and participants who consumed 25% or more had 2.75 (95% CI: 1.40-5.42) times greater risk of

CVD, after adjusting for confounders.

In addition to being a risk factor for GDM, PCOS is also a risk factor for CVD (101). A

systematic review and meta-analysis of controlled observational studies that examined the

association between PCOS and CHD reported a 2-fold increase in risk for CHD or stroke in

women with PCOS compared to women without PCOS [RR=2.02 (95% CI: 1.47-2.76)] (101).

The risk of developing CHD and stroke remained elevated after pooling two studies with risk

estimates adjusted for BMI, which suggests that the increased risk of CHD and stroke associated

with PCOS is not completely related to a higher BMI in women with PCOS [RR=1.55 (95% CI:

1.27-1.89)] (101).

Pre-eclampsia is a condition that manifests during pregnancy and is characterized by high

blood pressure, fluid retention, and proteinuria. Women with GDM are at higher risk of

developing pre-eclampsia (102). Pre-eclampsia is known to be a risk factor for CVD and may be

linked through common pathogenesis based on shared risk factors or through endothelial damage

that then in turn contributes to later CVD (103). A meta-analysis estimated that the RR for IHD

was 2.16 (95% CI: 1.86-2.52), for stroke was 1.81 (95% CI: 1.45-2.27), and for venous

thromboembolism was 1.79 (95% CI: 1.37-2.33) in women with pre-eclampsia compared to

women without, after a median of 11.7 years, 10.4 years, and 4.7 years of respective follow-up

(104). Likewise, a more recent meta-analysis assessed the risk of heart disease and stroke

separately but found similar results. This meta-analysis estimated that women diagnosed with

pre-eclampsia are at 128% (95% CI: 87%-178%) greater risk of fatal or diagnosed CVD and are

at 76% (95% CI: 43%-1.21%) greater risk of cerebrovascular disease (stroke) compared to women who did not have pre-eclampsia (105).

The association between hypertension and CVD has been examined in many epidemiological studies and the result of a strong, continuous, positive association has been consistent in both sexes, among various age categories, and within different races and ethnicities (106). The association between hypertension and CVD also extends to lower ranges of blood pressures that are termed prehypertension (107). A meta-analysis pooled data from 18 prospective cohort studies that included a total of 468,561 participants and reported that participants with prehypertension had an estimated 55% (95% CI: 41%-71%) higher risk of CVD, after adjustment for multiple cardiovascular risk factors when compared to participants who were normotensive (107). When IHD and stroke were examined separately, participants with prehypertension had an estimated 50% higher risk of IHD (95% CI: 30%-74%) and an estimated 71% higher risk of stroke (95% CI: 55%-89%) compared to participants who were normotensive (107). These findings emphasize that the association between hypertension and CVD is indeed strong, continuous, and positive.

Increasing parity may be associated with CVD in addition to GDM (108-110). A pan-European case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study estimated the association between parity and the risk of developing CHD and found that, compared to nulliparous women, multiparous women had 19% higher risk of developing CHD (95% CI: 1%-41%) after adjustment for age at study entry and centre, level of attained education, smoking status, high blood pressure, high-density lipoprotein cholesterol, total cholesterol, history of diabetes mellitus, and BMI (108). Women who gave birth five or more times were at even greater risk of developing CHD compared to nulliparous women

[adjusted hazard ratio (aHR)= 1.95 (95% CI: 1.19-3.20)] (108). A dose-response meta-analysis of cohort studies found that a J-shaped relationship exists between parity number and CVD mortality, where the risk of CVD mortality increases once the parity number reaches four live births (109).

Cholesterol is one of the substances that composes atherosclerotic plaques in artery walls, which leads to CVD (73). A meta-analysis aiming to summarize evidence on the effects of total serum (or circulating) cholesterol on the risk of CHD and stroke estimated a 20% (95% CI: 16%-24%) increase in CHD risk per 1 mmol/L higher concentration of total cholesterol in women; however, the risk of stroke was not significantly associated with total cholesterol (111). Specifically, low levels of high density lipoprotein (HDL), also known as "the good cholesterol", and high levels of triglycerides tend to be stronger predictors of CVD mortality than high levels of LDL. Individuals with HDL levels of < 1.30 mmol/L had a 74% (95% CI: 10%-175%) increase in risk of CVD death after adjustment for other CVD risk factors (112). Adults with triglyceride levels ≥ 4.50 mmol/L are at 3.44 times (95% CI: 1.65-7.20) the risk of CVD compared to those with triglyceride levels of < 2.25 mmol/L (112).

The relationship between race and ethnicity and CVD is complex and consists of interactions between biological, social, political, and environmental factors. Racial and ethnic minority populations experience disproportionate rates of CVD (113). For example, Indigenous peoples in Canada experience a greater prevalence of CVD compared to Canadians of European descent and have greater prevalence of traditional CVD risk factors such as hypertension, diabetes, smoking, and hyperlipidaemia (114). Many of these risk factors also interact with SES. Low SES, which is typically measured through proxy variables such as years of formal education, highest educational attainment, occupation, and housing characteristics (115), is

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associated with many adverse health outcomes including CVD (116). This association may be partly attributable to factors such as poor adherence to treatment such as medication and smoking cessation plans, reduced access to health care (117), and poor health behaviours concerning cigarette smoking, alcohol consumption, physical activity, and nutrition (116). For example, dietary intake of fruits and vegetables, a necessary component of a healthy diet, is positively associated with neighborhood SES (118). Perceived unaffordability and inaccessibility of these foods has been cited as a barrier to consumption for low-income women (119).

A sex difference in CVD risk attributable to SES may also exist. Backholer et al. (120) conducted a meta-analysis and found that the association of SES with CHD and CVD was significantly stronger in women than in men. Women with the lowest level of educational attainment had 34% (95% CI: 9%-63%) greater risk of CHD compared to men with the same level of education attainment after adjusting for major CVD risk factors (120). Women with the lowest educational attainment level also had an 18% greater risk of CVD compared to men with the same level of education (120). Meanwhile, there was no evidence of sex difference in the excess risk of stroke associated with either educational attainment levels or alternative measures of SES such as area deprivation, occupation, and income. Disproportionate risk of CVD in women with low SES compared to men with low SES can likely be partly attributed to a lack of awareness of CVD symptoms and a delay in seeking treatment due to barriers of access (121).

Family history of CVD, especially CHD, has commonly been cited in the literature as a risk factor for future CVD; however, caution is warranted when interpreting results as measuring the magnitude of risk is complicated by methodological factors such as ascertainment of this information and varying definitions of family history and CVD outcomes. In a multivariable

analysis of traditional Framingham risk factors, family history of CHD was predictive of major adverse cardiac events in women [hazard ratio (HR): 2.07 (95% CI: 1.32-3.24)] (122).

A meta-analysis including 858,507 subjects found that the RR of developing incident CHD associated with T2DM was 44% (95% CI: 27%-63%) greater in females than in males (9). Similarly, Huxley et al. (123) documented a 46% (95% CI: 14%-88%) excess risk of fatal CHD in females with T2DM compared to males with T2DM in an older meta-analysis that included studies with adjustments for multiple potential confounders. Pre-menopausal women with T2DM have approximately the same risk of CHD as men of the same age with T2DM; therefore, it appears that T2DM eliminates the protective advantage associated with being female in pre-menopausal women (123). Type 2 diabetes mellitus is one of the main variables in the present study and evidence for its association with CVD will be reviewed in depth in section 2.4.2.

2.3 Established Risk Factors for Type 2 Diabetes Mellitus

The purpose of this section is to present some of the risk factors for T2DM and to highlight that many of them overlap as risk factors for both GDM and CVD. As a result, most of these risk factors were discussed in depth in sections 2.1.3 and 2.2.3. A recent umbrella review of meta-analyses identified increasing BMI, unhealthy diet, lower educational status, physical inactivity, smoking, and hypertension as risk factors for T2DM (124). In addition to these risk factors, Diabetes Canada also included age of 40 years or greater, first-degree relative with T2DM, member of high-risk population (e.g., African, Arab, Asian, Hispanic, Indigenous, or South African descent), and presence of PCOS and acanthosis nigricans as risk factors in their 2018 Clinical Practice Guidelines (5). Relationships between GDM, T2DM, CVD, and their risk factors are outlined in simplified directed acyclic graphs in figures 2-1, 2-2, and 2-3.

2.4 Biological Plausibility for the Association between Gestational Diabetes Mellitus and Cardiovascular Disease

A causal link between GDM and CVD is biologically plausible and is supported by physiological changes to the mother's cardiometabolic system during pregnancy. While the clinically important symptoms of GDM usually disappear after pregnancy, permanent adverse effects on the cardiovascular system could lead to higher risk of maternal CVD later in life. Women with prior GDM may be more likely to develop T2DM and CVD later in life relative to women without a history of GDM (7). It is likely that GDM negatively affects endothelial structure and function, paving the way for the development of atherosclerosis, which plays a central role in the pathophysiology of CVD.

Ultrasound imaging of carotid arteries is often conducted to measure common carotid intima-media thickness (ccIMT) and plaque, both used as proxy measures of CVD. Common carotid intima-media thickness is used to measure early atherosclerosis and this measurement has been found to be a strong predictor of MI, particularly in women (125). Several studies in recent years have been published on ccIMT in women with history of GDM. Common carotid intima-media thickness was found to be elevated or similar in women diagnosed with GDM compared to those who were not in cross-sectional studies (126,127). However, these cross-sectional studies were unable to answer whether a history of GDM contributed to early atherosclerosis risk independent of T2DM and metabolic syndrome as screening for these conditions before or after the pregnancy did not occur and, therefore, risk of these conditions preceding CVD could not be assessed. The prospective CARDIA study addressed this issue and found that a history of GDM is associated with early subclinical atherosclerosis before the onset of T2DM and metabolic syndrome in the 20 years following delivery independent of pre-pregnancy obesity, race, parity, and age (128). Among women who did not develop T2DM or metabolic syndrome during the 20-

year follow-up period, the mean ccIMT was 0.023 mm (95% CI: 0.001-0.045, p-value: 0.039) greater for women with a history of GDM than women without a history of GDM after adjustment for the confounders mentioned above and pre-pregnancy BMI. In contrast, the mean ccIMT did not differ by GDM history among women who developed T2DM or metabolic syndrome, and therefore the results indicate that history of GDM may be an indicator of early atherosclerosis independent of pre-pregnancy BMI and T2DM (128).

Inflammation has also been implicated in the development and progression of CVD, most notably through its association with atherosclerosis (129). Several inflammatory molecules likely play a role in these processes and are associated with GDM, including tumor necrosis factor-α, which is inversely correlated with insulin secretion, interleukin-6 and CRP, as well as the anti-inflammatory molecule adiponectin. Markers of endothelial dysfunction like E-selectin, ICAM-1, vascular cellular adhesion molecule-1 (VCAM-1), and pentraxin 3 are also thought to play a major role in the pathogenesis of vascular disease (130,131). These adhesion molecules aid in the formation of atherosclerotic plaques after being expressed on the surface of vascular endothelial cells in response to inflammatory mediators and may serve as markers for the severity of atherosclerosis (130,131).

Inflammation may also play a role in the pathogenesis of GDM as this condition has been linked to the activation of a network of inflammatory signaling pathways, although the exact mechanism contributing to GDM is still unknown. During pregnancy, GDM contributes to down-regulation of adiponectin and up-regulation of pro-inflammatory markers such as interleukin-6, E-selectin, and VCAM-1 which contribute to insulin resistance (132,133). E-selectin and ICAM-1 levels, which are elevated in both normal and GDM pregnancies, remained elevated after delivery only in women whose pregnancies were complicated by GDM despite

blood glucose tolerance returning to normal (134). Persistent elevation of these molecules suggests that GDM may be associated with vascular injury. Collectively, these data are indicative of an underlying defect in women with prior GDM that is ultimately contributing to increased CVD risk.

Oxidative stress attributable to overproduction of free radicals and defects in antioxidant defence mechanisms may also contribute to the association between GDM and CVD (135). A hyperglycaemic environment created during GDM induces oxidative stress and cell and tissue damage through several biochemical pathways that can result in endothelial dysfunction in the mother after GDM (135). Oxidative stress generated because of GDM may activate the expression of E-selectin, VCAM-1, and ICAM-1 which, as previously discussed, contributes to atherosclerosis and subsequently CVD (135).

Furthermore, the risk of postpartum CVD may also extend to milder degrees of antepartum glucose intolerance, which suggests a biological gradient may exist, thus meeting an additional criterion on Hill's list of criteria for causation (136). As previously mentioned, pregnant women are usually screened for GDM by being administered a 50 g GCT. Those who have an abnormal result on the GCT are then given a diagnostic OGTT. In their study of 71,831 women with an abnormal result of the GCT that did not meet the diagnostic criteria for GDM in the subsequent OGTT, Retnakaran et al. (136) estimated that women with any degree of abnormal glucose homeostasis on antepartum screening had 19% (95% CI: 2%-39%) increased risk of CVD compared to women with no dysglycemia after 12.3 years of median follow-up time. This raises the possibility that the risk of CVD associated with glucose intolerance extends beyond the cutoff for a diagnosis for GDM. Decreased brachial artery flow-mediated dilation, indicative of endothelial dysfunction and early atherosclerosis, may be the factor linking CVD

with mild glucose intolerance. A previous study supported the theory that glucose intolerance in pregnant women, including both mild cases and those severe enough to meet the criteria of GDM, is closely related to endothelial dysfunction (137). Paradisi et al. (137) demonstrated that flow-mediated dilation in pregnant women with impaired glucose tolerance was 70% of that seen in control subjects, decreasing to 38% in women with diagnosed GDM. They also established that flow-mediated dilation decreases linearly with increases in glucose area (mg/dL) (a measurement that is indicative of degree of glucose intolerance) (137).

2.5 Literature Review of Existing Evidence on the Associations among Gestational Diabetes Mellitus, Type 2 Diabetes Mellitus, and Cardiovascular Disease

Associations between GDM and between T2DM and T2DM and CVD have been well-established in the literature. GDM and T2DM likely have shared pathophysiology due to an underlying defect in pancreatic β-cell function that surfaces during pregnancy, resulting in a diagnosis of GDM. The ongoing deterioration of function of the pancreatic β-cell and concurrent insulin resistance can result in a diagnosis of T2DM after pregnancy. More recent published studies have focused on the association between GDM and future risk of CVD and have found that women who have had GDM are at greater risk of developing CVD in the future. However, it has not been established whether the increased risk of CVD associated with GDM is independent of T2DM. A simplified directed acyclic graph of the potential relationship among these variables is provided in Figure 1. The existing literature on the associations among GDM, T2DM, and CVD, as well as the inherent limitations of these studies, will be discussed in the following section.

2.5.1 Gestational Diabetes Mellitus and Type 2 Diabetes Mellitus

The glucose intolerance associated with GDM is restored to non-pregnancy levels shortly after delivery; however, women affected by GDM remain at high risk of developing T2DM later in life. The RR of this association ranged from 1.30 (95% CI: 0.46-3.78) to 47.3 (95% CI: 2.95-757.28) in previous studies reviewed in a meta-analysis conducted by Bellamy and colleagues (8). This meta-analysis, published in 2009, synthesized data from 20 studies, representing 675,455 women and 10,859 incident cases of T2DM, and estimated that the risk of developing T2DM in women with GDM is increased 7.4-fold (95% CI: 4.8-11.5) (8). The mean follow-up period ranged from six weeks to 28 years (8). The magnitude of the association between GDM and T2DM as well as shared risk factors such as obesity, lack of physical activity, race/ethnicity, and family history of T2DM suggest the two conditions may have shared pathophysiology. Gestational diabetes identifies women with a defect in β-cell function, a dysfunction that predisposes some women to T2DM in the following years (8,13).

The meta-analysis conducted by Bellamy et al., (8) while valuable in that it synthesizes data from many studies, has fundamental flaws. Bellamy et al. (8) calculated and pooled unadjusted RRs with 95% CI for each study instead of including adjusted estimates. It is possible that the association between GDM and T2DM is overestimated in each of these studies since some of the increased risk of T2DM may be explained by confounders. By pooling the unadjusted risks, Bellamy et al. (8) may have propagated the overestimation from each of these studies. Additionally, the studies included in this meta-analysis had a wide range in reported RRs. Factors that contribute to this heterogeneity could include differences in screening guidelines followed (i.e., screening was population-based or risk factor-based), GDM diagnostic

criteria, and duration of follow-up. Bellamy et al. (8) should have conducted a sensitivity analysis to identify important sources of the heterogeneity in results across the included studies.

2.5.2 Type 2 Diabetes Mellitus and Cardiovascular Disease

It is widely accepted in the literature that individuals with T2DM are at increased risk of CVD and its associated complications and that this risk is greater among women than men. Peters et al. (9) included data from 64 cohorts, including 858,507 individuals in their meta-analysis and found that the risk of incident CHD among women with T2DM was 182% (95% CI: 135%-238%) greater than among women without T2DM and that the excess risk of CHD associated with T2DM was significantly higher in women than in men [RR=1.44 (95% CI: 1.27-1.63)]. The results from another meta-analysis using stroke as the primary outcome instead of CHD demonstrated similar results as women with T2DM had significantly greater risk of stroke compared to women without T2DM [RR=2.28 (95% CI: 2.93-2.69)] and that women with T2DM had a greater risk of stroke compared to men [RR=1.27 (95% CI: 1.10-1.46)] (10). Possible explanations for these sex disparities are that women may experience more CVD risk factors than men and may be exposed to these risk factors longer (10). Women may also have greater adiposity levels and may experience undertreatment compared to men (10).

Myocardial infarction and stroke are the most commonly reported primary outcomes studied in association with T2DM, but there is a gap in knowledge of how T2DM affects other acute and chronic cardiovascular manifestations. Shah et al. (138) addressed this gap by assessing a wider range of cardiovascular outcomes and found that T2DM was positively associated with PAD (adjusted HR=2.98 (95% CI: 2.76-3.22)], ischaemic stroke [HR=1.72 (95% CI: 1.52-1.95)], stable angina [HR=1.62 (95% CI: 1.49-1.77)], heart failure [HR=1.56 (95% CI: 1.45-1.69)], and non-fatal MI [HR=1.54 (95% CI: 1.42-1.67)], but was inversely associated with

abdominal aortic aneurysm [HR=0.46 (95% CI: 0.35-0.59)] and subarachnoid haemorrhage [HR=0.48 (95% CI: 0.26-0.89)]. Type 2 diabetes mellitus was not associated with arrhythmia or sudden cardiac death [(HR=0.95 (95% CI: 0.76-1.19)] (138).

2.5.3 Gestational Diabetes Mellitus and Cardiovascular Disease

The adverse maternal postpartum outcomes associated with GDM are well known, as well as the increased risk of cardiometabolic risk factors and disorders; however, the extent of the association between GDM and the risk of CVD and whether the association is independent of traditional cardiovascular risk factors including T2DM remains inconclusive in the literature.

The effect of GDM on CVD may be mediated by T2DM, but GDM may still affect CVD independently from T2DM. The association between GDM and CVD can be evaluated by assessing three paths: 1) the direct effect, which is the effect of GDM unexplained by T2DM; 2) the indirect effect, which is the effect of GDM on CVD that is explained by T2DM, and 3) the total effect, which is the sum of the direct effect and indirect effect. The total effect of GDM on CVD and the potential for mediation by T2DM found in the existing literature will be discussed below.

Total Effect of Gestational Diabetes and Cardiovascular Disease

A search of the literature yielded twelve studies, summarized in table 2-1, that investigated whether women with GDM have increased risk of CVD (7,11,12,136,139-146). All these studies reported positive associations between GDM and CVD, but these studies differed in sample size, study population, follow-up time, and the confounders included in their statistical models (7,11,12,136,139-144,146). These studies also differed in how they ascertained GDM.

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For example, Retnakaran et al. (12) selected a random pregnancy for those women with multiple pregnancies, while Kaul et al. (141) ascertained GDM status from the index pregnancy, defined as the first pregnancy of each woman that occurred during the study period. Definitions of CVD were also inconsistent among these studies as some studies considered cardiac procedures to be indicative of CVD events, whereas others did not. The definitions of CVD in these studies are summarized in table 2-2.

A recent systematic review and meta-analysis included seven of the twelve studies identified in the present literature review (6). These seven cohort studies included a total of 3,417,020 women and 14,146 incident CVD events. The pooled estimate suggested that women with previous GDM had an elevated risk of developing CVD compared to those without previous GDM [RR: 1.74 (95% CI: 1.28-2.35, I^2=95.7%)].

Two of these studies used a cross-sectional design with a small sample size (7,144). Carr et al. (7) used self-reported data on history of GDM to examine whether it was associated with an increased risk of CVD among 995 women with first degree relatives with T2DM and found that, compared to women without history of GDM, women with prior GDM were more likely to have CVD, after adjustment for age and menopausal status and clustering on the proband [aOR=1.85 (95% CI: 1.21–2.82)]. Shostrom et al. (144) included 8,127 women who participated in the 2007-2014 cycles of the National Health and Nutrition Examination Survey (NHANES) in the United States and found that a history of GDM was associated with 63% higher odds of CVD after adjustment for demographic, socioeconomic, and lifestyle factors [aOR=1.63 (95% CI: 1.02-2.62)]. In a cross-sectional study, all factors (e.g., the exposure, outcome, and confounders) are measured simultaneously. As a result, it is difficult to establish temporal relationships, and this can lead to biased estimates of the association between GDM and CVD. Cross-sectional studies

also measure prevalent cases of the outcome rather than incident cases and, therefore, the results of these studies are susceptible to survival bias. A history of GDM and CVD diagnosis in these studies, along with information on confounders, were self-reported in these studies and this may have resulted in recall bias and misclassification bias (7,144).

As mentioned in sections 2.1.3 and 2.2.3, body mass is associated with both GDM and CVD and is, therefore, a likely confounder of the association between these two diseases. Only four of these studies adjusted for or stratified on body mass (139,142-144). In the study conducted by Shostrom et al. (144), an association between GDM and CVD was found after adjusting for various confounders [aOR=1.63 (95% CI: 1.02-2.62)]. Adjusting for body mass in addition to the various confounders attenuated the association between GDM and CVD [aOR=1.52 (95% CI: 0.95-2.44)] (144). This change indicates that body mass is an important confounder of the association between GDM and CVD and should be adjusted for in statistical models.

Another issue in previous studies is short length of follow-up time. Follow-up in these studies was mainly limited to ten to twelve years, with the exception of two studies (Table 2-1). This may not have been sufficient time for these women to develop CVD and, therefore, the persistence of the association with later onset of CVD has not been adequately examined.

Five studies examined the association between GDM and the risk of stroke and IHD as separate end points (7,140,143,145,146). All five studies found that GDM was positively associated with IHD (7,140,143,145,146), but only one study found that GDM was associated with stroke (146). Stroke was a relatively rare outcome in these studies and, therefore, it is possible that these studies were underpowered with respect to stroke.

Mediation by Type 2 Diabetes Mellitus

Traditionally, mediation analysis has been applied in studies using survival data by comparing two Cox regression models, one with adjustment for the mediator and one without this adjustment, to estimate the direct effect of the exposure. In this approach, the proportion of the total effect of the exposure on the outcome of interest explained by the mediator is obtained by calculating a ratio of the unadjusted hazard ratio (uHR) to the aHR. A ratio other than 1:1 indicates that some of the effect of the exposure can be attributed to mediation through the mediator. This approach is, however, problematic as it is prone to bias and can lead to flawed results (147). The three main sources of bias that exist in the traditional approach to mediation analysis are: mediator-outcome confounding, exposure-mediator interaction, and mediator-outcome confounding affected by the exposure (147). According to Lange (148), the traditional approach to mediation analysis is mathematically problematic as the proportional hazards assumption in both the unadjusted and adjusted Cox models cannot be satisfied. Some studies have also examined the joint association between GDM and T2DM in relation to CVD to assess mediation.

Five studies used the traditional approach to mediation analysis to assess whether T2DM mediated the association between GDM and CVD and found conflicting results (7,11,136,139,140). Three of the five studies found that T2DM fully attenuated the association between GDM and CVD (11,136,139), while the other two studies found that the association between GDM and CVD remained after adjustment for T2DM (7,140).

Two previous studies evaluated the single and joint associations of GDM and T2DM on the risk of developing CVD later in life as a way of exploring mediation (12,143). To do this,

women with exposure to GDM were further categorized by progression to T2DM and four groups were created: no GDM and no progression to T2DM, GDM and no progression to T2DM, no GDM and progression to T2DM, and GDM and progression to T2DM. Retnakaran and colleagues (12) found that all groups exhibited an increased risk of CVD when compared to the reference group of no GDM and no T2DM, after adjustment for age, income, and region of residence. These findings suggest that women with GDM in whom T2DM does not develop are still at increased risk of developing CVD later in life; however, these findings are not consistent with what was found by Tobias and colleagues (143). Tobias and colleagues found that women with a history of GDM but who did not develop T2DM did not experience an elevated CVD risk after adjusting for confounders, including pre-pregnancy BMI [aHR=1.30 (95% CI: 0.99-1.71)]. Women who did not have GDM but developed T2DM and women who had GDM and developed T2DM had greater than a four-fold increase in CVD risk [aHR=4.48 (95% CI: 2.25-8.91), aHR=4.04 (95% CI: 1.96-8.36), respectively].

Overall, the estimates in studies that have attempted to assess mediation of the association between GDM and CVD by T2DM are affected by three main sources of bias: T2DM-CVD confounding, GDM-T2DM interaction, and T2DM-CVD confounding affected by GDM, which may have led to inaccurate results and flawed conclusions. These studies were also unable to provide an estimate of the proportion mediated by T2DM as this measure can only be obtained with the traditional methods for assessing mediation when linear regression is used.
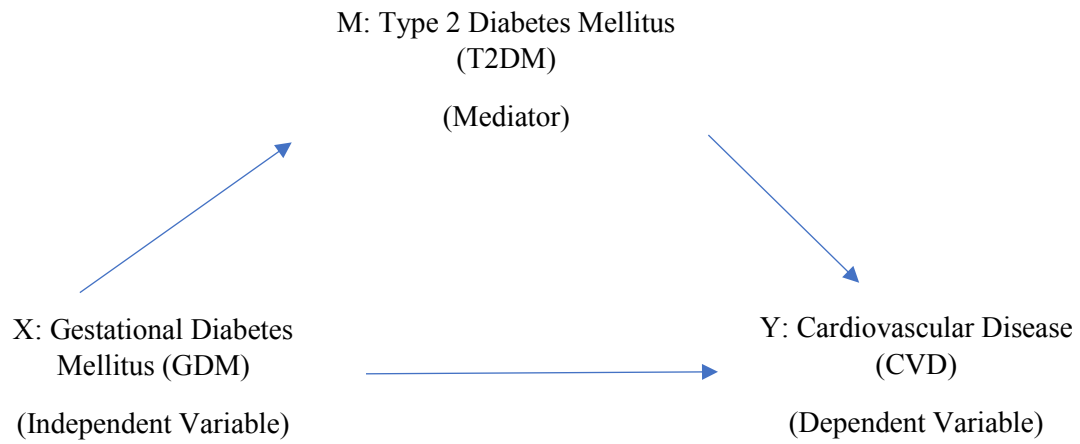
Figure 2-1. A simplified directed acyclic graph of the potential relationships among gestational diabetes mellitus, type 2 diabetes mellitus, and cardiovascular disease
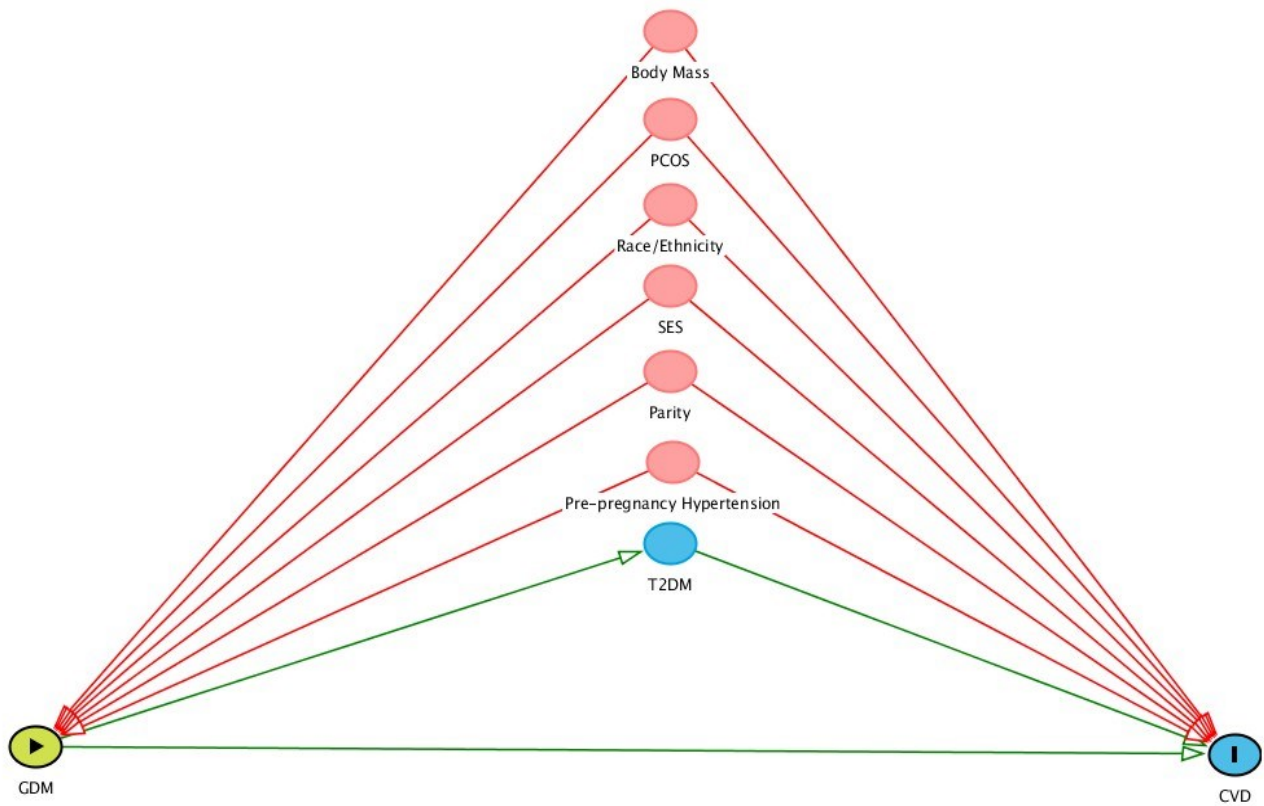
Figure 2-2. A simplified directed acyclic graph of the potential relationships among gestational diabetes mellitus, type 2 diabetes mellitus, cardiovascular disease, including potential confounders of the association between gestational diabetes mellitus and cardiovascular disease
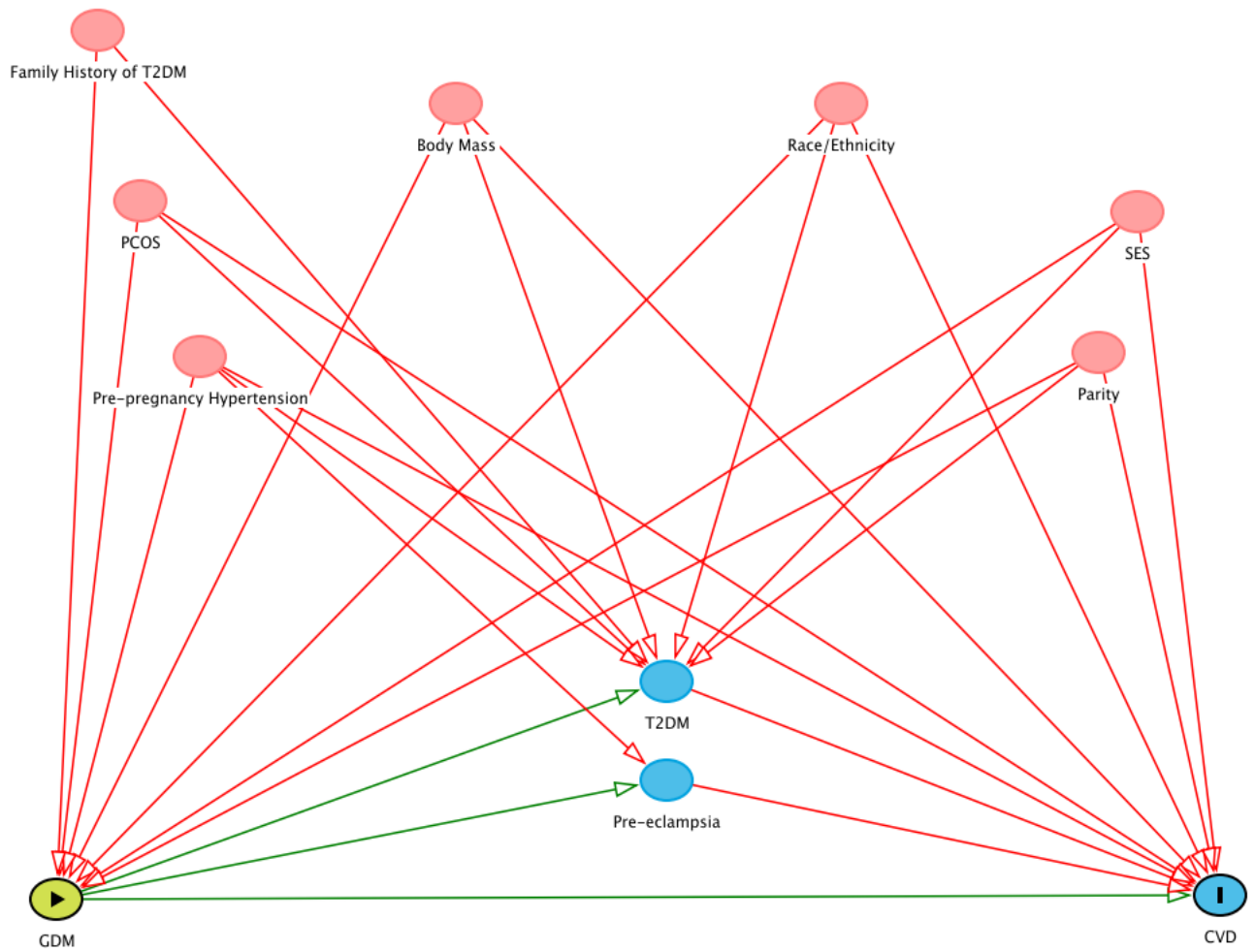
Figure 2-3. A simplified directed acyclic graph of the potential relationships among gestational diabetes mellitus, type 2 diabetes mellitus, cardiovascular disease, and other variables

Table 2-1. Characteristics of studies estimating the association between gestational diabetes mellitus and cardiovascular disease

Author, Year	Country	Study Design	N	Follow-Up	Effect Measure (95% CI)	Confounders/ Covariates
Carr et al., 2006 (7)	United States	Cross-sectional	994	N/A	**aOR (model 1)** 1.66 (1.07-2.57) **aOR (model 2)** 1.56 (1.002–2.43)	model 1: age, menopausal status, clustering on the proband, race/ethnicity model 2: proband status, T2DM
Fadl et al., 2014 (139)	Sweden	Case-control	2639	Mean: 9.1 years	**uOR** 2.19 (1.59–3.01) **aOR** 1.51 (1.07–2.14)	chronic hypertension, smoking, BMI, ethnicity, education level, parity
Goueslard et al., 2016 (140)	France	Retrospective cohort	1,515,387	Total: 7 years	**aOR (model 1)** 1.39 (1.21–1.59) **aOR (model 2)** 1.25 (1.09–1.43)	model 1: age model 2: age, obesity, subsequent T2DM and hypertensive diseases during pregnancy
Kaul et al., 2014 (141)	Canada	Retrospective cohort	240,083	Median: 5.3 years	**aHR CVD** GDM only: 1.4 (1.0-1.9) GDM & overweight: 2.1 (1.1-3.5)	maternal age, pre-eclampsia, parity, smoking during pregnancy, ethnicity, socioeconomic status, and GDM during a subsequent pregnancy
Kessous et al., 2013 (142)	Israel	Retrospective cohort	47,909	Total: >10 years	**aOR simple CV events** 2.7 (2.4-3.1) **aOR complex CV events** 1.7 (0.9-3.3) **aHR CV hospitalizations (Cox model)** 2.6 (2.3-2.9)	ethnicity, age Cox model: pre-eclampsia, obesity

Author, Year	Country	Study Design	N	Follow-Up	Effect Measure (95% CI)	Confounders/ Covariates
Retnakaran and Shah, 2009 (136)	Canada	Retrospective cohort	435,696	Median: 12.3 years	**aHR (model A)** 1.66 (1.30–2.13) **aHR (model B)** 1.25 (0.96–1.62)	Model A: age, year of delivery, rural residence, income, comorbidity, pre-existing hypertension and gestational hypertension Model B: model A + development of T2DM
Retnakaran and Shah, 2017 (12)	Canada	Retrospective cohort	1,515,079	Median: 10.0 years	**aHR CVD events** GDM/DM: 2.82 (2.41–3.30) GDM/no DM: 1.30 (1.07–1.59) no GDM/DM: 2.01 (1.82-2.20) **aHR CAD events** GDM/DM: 3.54 (2.96–4.23) GDM/no DM: 1.41 (1.11–1.80) no GDM/DM: 2.25 (2.01-2.52)	age, income, and region of residence
Shah et al., 2008 (11)	Canada	Retrospective cohort	89,453	Median: 11.5 years	**uHR** 1.71 (1.08–2.69) **aHR** 1.13 (0.67–1.89)	subsequent T2DM
Shostrom et al., 2017 (144)	United States	Cross-sectional	8127	N/A	**aOR (model 1)** 1.60 (1.02-2.53) **aOR (model 2)** 1.63 (1.02-2.62) **aOR (model 3)** 1.52 (0.95-2.44)	model 1: age model 2: model 1 + race/ethnicity, education, ratio of family income to poverty, smoking status, alcohol intake, physical activity, and total energy intake model 3: model 2 plus BMI

Author, Year	Country	Study Design	N	Follow-Up	Effect Measure (95% CI)	Confounders/ Covariates
Tobias et al., 2017 (143)	United States	Prospective cohort	89,479	Median: 25.7 years	**aHR (model 1)** 1.60 (1.26-2.04) **aHR (model 2)** 1.43 (1.12-1.81)	model 1: age model 2: age + years since first birth age, menopausal status, current hormone therapy use, white race/ethnicity, family history of MI or stroke, history of pregnancy hypertensive disorders, pre-pregnancy BMI, and parity
Daly et al., 2018 (145)	United Kingdom	Retrospective cohort	46,399	Median: 2.9 years	**aIRR (IHD)** 2.78 (1.37-5.66) **aIRR (Stroke or TIA)** 0.95 (0.51-1.77)	age, Townsend quintile, BMI, smoking, prescribed lipid-lowering medication, hypertension
McKenzie-Sampson et al., 2018 (146)	Canada	Retrospective cohort	1,070,667	Maximum: 25.2 years	**uHR** 1.74 (1.68-1.81) **aHR** 1.75 (1.69-1.81)	baseline age, parity, time period, socioeconomic deprivation

aHR, adjusted hazard ratio; aIRR, adjusted incidence rate ratio; aOR. adjusted odds ratio; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; IHD, ischaemic heart disease; MI, myocardial infarction; T2DM, type 2 diabetes mellitus; TIA, transient ischaemic attack; uOR, unadjusted odds ratio

Table 2-2. Definitions of cardiovascular disease in studies examining the association between gestational diabetes mellitus and cardiovascular disease

Author, Year	Definition of CVD Outcome
Carr et al., 2006 (7)	Questionnaire regarding history of CAD & stroke
Fadl et al., 2014 (139)	Inpatient diagnosis or death identifying IHD, ischaemic stroke, atherosclerosis, or peripheral vascular disease
Goueslard et al., 2016 (140)	Angina pectoris, MI, stroke, heart bypass surgery, coronary angioplasty, carotid endarterectomy and fibrinolysis
Kaul et al., 2014 (141)	IHD, cerebrovascular disease
Kessous et al., 2013 (142)	Angina pectoris, congestive heart failure, and invasive and non-invasive cardiac procedures (e.g., insertion of a stent and a treadmill stress test)
Retnakaran and Shah, 2009 (136)	Admission to hospital for acute MI, CABG, coronary angioplasty, stroke, or carotid endarterectomy
Retnakaran and Shah, 2017 (12)	Primary: CVD event (hospitalization for MI, acute coronary syndrome, CABG, PCI, stroke, TIA, or carotid endarterectomy, CAD events)
Shah et al., 2008 (11)	Primary: hospitalization for acute MI, stroke, coronary artery bypass, coronary angioplasty, or carotid endarterectomy Secondary: hospitalization for acute MI, CABG, or coronary angioplasty
Shostrom et al. 2017 (144)	Self-reported CVD (interview asked about congestive heart failure, CHD, angina/angina pectoris, heart attack, stroke)
Tobias et al. 2017 (143)	Self-reported non-fatal or fatal MI or stroke confirmed by medical records
Daly et al., 2018 (145)	Clinical diagnosis of IHD and cerebrovascular disease (stroke or TIA)
McKenzie-Sampson et al., 2018 (146)	Hospitalization for IHD, MI, angina pectoris, cardiac arrest, heart failure, cardiomyopathy, valve disorders, aortic dissection, pulmonary embolism, ischaemic stroke, hemorrhagic stroke, atherosclerosis, and hypertension. Definition also included the following procedures: coronary angioplasty, CABG, valve surgery, aorta surgery, pacemaker insertion, angiography, cardiopulmonary resuscitation, and admission to an intensive care unit

CABG, coronary artery bypass graft; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; IHD, ischaemic heart disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; TIA, transient ischaemic attack

Chapter 3 OBJECTIVES

This study used administrative data to assess the relationship between GDM and CVD in mothers who gave birth between 1988 and 2012 in Nova Scotia. Specifically, the two primary objectives were to:

I. Quantify the risk of subsequent CVD associated with GDM;

II. Estimate the extent to which T2DM acts as a mediator of the association between GDM and CVD

The secondary objective was to:

I. Explore objectives I and II by type of CVD (i.e., IHD and stroke)

Chapter 4 METHODS

4.1 Overview of the Study Design

This study was conducted as an extension of an existing longitudinal retrospective cohort study on women who delivered children between 1988 and 2012 in Nova Scotia (Principal Investigators: Christy Woolcott and Linda Dodds, Perinatal Epidemiology Research Unit (PERU), IWK Health Centre). Information on GDM and potential confounders was obtained from the NSAPD, and outcome information on T2DM and CVD was derived through linkage with the following administrative health databases: physician billing, hospital discharge records, insured patient registry, and vital statistics. Follow-up of eligible women started from their last pregnancy and used information from these administrative databases between 1989 and 2014. The aim of the parent study was to generate risk prediction models for CVD using pregnancy characteristics such as GDM. The present study took on a more etiological aim to fill gaps in the literature pertaining to the association between GDM and CVD that complements and informs the analysis conducted in the parent study.

4.2 Ethical Considerations

Linkage of the databases was carried out by a third party (Health Data Nova Scotia (HDNS)) with encrypted identifiers using a cross-walk file with the Nova Scotia Health Card number maintained by Medavie Blue Cross. De-identified data was stored on and accessed through servers of HDNS. The research protocol of the parent study was submitted to and received approval from the Joint Data Access Committee of the Reproductive Care Program (RCP) (File # JDAC63, 19 March 2013), HDNS Data Access Committee (File # 2012-CAW-001, 04 December 2013) and the Research Ethics Board of the IWK Health Centre (File #

1013732, 28 March 2013). I applied to have amendments made to the approvals of the parent research protocol, and this study was included as an extension. All procedures performed in this study were in accordance with the ethical standards of the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, December 2014*. I oriented myself with these ethical standards when I completed the online Tri-Council Policy Statement 2 tutorial. I also participated in in-service training regarding privacy from the two data custodians.

4.3 Population and Study Eligibility

The target population in this study was Nova Scotian women who gave birth to infants with birthweight ≥ 500 g and gestational age ≥ 20 weeks between 1988 and 2012. Women were excluded if (a) their first pregnancy was not captured in the NSAPD, (b) they had a pre-existing diagnosis of type 1 diabetes mellitus (T1DM) or T2DM, (c) they could not be followed for at least two years using the administrative databases, or (d) they had a CVD event prior to their first pregnancy. A woman who was diagnosed with T2DM up to six months after a delivery in which GDM was coded was considered as having unrecognized pre-existing diabetes and was also excluded from the analysis.

4.4 Sources of Data

Nova Scotia Atlee Perinatal Database (NSAPD)

Maternal exposure information, whether mothers were, or were not, diagnosed with GDM during their pregnancies between 1988 and 2012, was derived from the NSAPD, as was information on potential confounders. The NSAPD is a high-quality database administered by the RCP (149,150). It contains records of all births to mothers residing in Nova Scotia since 1988, including those who delivered in New Brunswick, and whose infants were ≥ 500 g or ≥ 20

weeks gestational age (149). Recording of home births in this database began in 2009, corresponding to the introduction of regulated midwifery in the province. Home births represent < 0.1% of all births in Nova Scotia (Irene Gagnon, Reproductive Care Program of Nova Scotia, Personal Communication).

The NSAPD collects information from standardized forms such as the Prenatal Record, Discharge Summary, and Maternal Assessment Form. Trained health records personnel enter this information in the database. Specifically, the NSAPD contains socio-demographic variables, information on procedures and interventions undergone, maternal and newborn diagnoses, and morbidity and mortality records for everyone included. Unique identifiers are associated with every mother that enable the identification of successive pregnancies. Deliveries that are missing (e.g., those occurring outside of Nova Scotia) from the NSAPD can be identified using parity information recorded for each delivery. The NSAPD regularly undergoes data quality assurance checks, including re-abstraction and validation studies, to ensure it is reliable and of high quality (151).

Administrative health services databases

Administrative health services databases were the source of data for the occurrence of maternal T2DM and CVD and include physician billing, hospital DAD, insured patient registry, and vital statistics. Follow-up in these databases was from the woman's last pregnancy to 2014. These databases are described in the following sections.

Physician Billings (Nova Scotia Medical Services Insurance)

Insured services provided by physicians that are paid for by the provincial health care system have been captured in the Medical Services Insurance physician billing database since 1989. This database contains diagnostic codes in the International Classification of Diseases,

Ninth Revision, Clinical Modification (ICD-9) format and procedure codes for each service rendered. This database had the capacity to capture only one diagnosis per visit between 1989 and 1997, increasing to three diagnoses per visit thereafter.

Hospital Discharge Abstract Database

The DAD, maintained by the Canadian Institute for Health Information, contains administrative, clinical, and demographic information on hospital discharges (including deaths, sign-outs, and transfers) for all individuals in Canada (except military) from 1995 onward. Between 1989 and 1994, the Admissions, Separations, and Day Surgeries database captured hospital data in Nova Scotia. Both the Admissions, Separations, and Day Surgeries database and DAD contain patient demographics (e.g., age, gender, and location), attending physicians, diagnoses and procedures performed. The DAD contains additional information on service transfers while in hospital, specialty services, and case complexity (e.g., resource intensity weight). These databases contain diagnosis codes in the ICD-9 format between 1989 and 2000 and codes in the ICD-10-CA format between 2001 and 2014. Procedure codes were in the ICD-9 format until 2000 and thereafter, were in the Canadian Classification of Interventions format. Up to seven diagnoses per visit were captured between 1989 and 1995, increasing to 16 diagnoses per visit between 1995 and 2001, and then to 25 diagnoses per visit in 2001 and remained at this number for the duration of the follow-up period in this study.

Insured Patient Registry

Longitudinal information regarding the entire population of insured beneficiaries of Nova Scotia healthcare services is contained in this registry. It was used to determine whether any mothers in the NSAPD cohort died or left the province and if they did, when.

Vital Statistics

The Vital Statistics database was used to verify a code of death in the insured patient registry and allowed a determination of whether the cause of death was due to CVD.

Method of Linkage

A unique Provincial Health Number is assigned to all individuals born in Nova Scotia and remains associated with the individual throughout their lifetime, regardless of whether they leave the province and return. Provincial Health Numbers are consistent across all data sources used in this study, although they have been encrypted in the administrative databases to maintain anonymity. The linkage for the parent study was performed by HDNS at Dalhousie University, whom the Government of Nova Scotia has provided the administrative health services databases. The integrity of the linkage was assessed by cross-referencing birth date and sex between the databases. Previous studies have been successful in linking perinatal data in the NSAPD to health services databases to determine diagnoses of medical conditions such as diabetes in mothers (152) and autism in offspring (153). For the present cohort, the linkage was successful for 94.8% of the women identified as being eligible from the NSAPD.

4.5 Variables and Confounders

4.5.1 Exposure Variable: Gestational Diabetes Mellitus

The main exposure of interest in this study was GDM and was obtained from the NSAPD. The Prenatal Record was the likely source for a code of GDM in the NSAPD. This form is filled out at each prenatal visit and includes lines for the results of the GCT and OGTT, and although exact laboratory values are not entered in the NSAPD, the diagnosis of GDM is recorded. A diagnosis of GDM can also be recorded on the admission forms at the time of

delivery, which is then coded in the NSAPD. The screening and diagnostic criteria used during the study period was described above in section 2.1.1.

4.5.2 Mediator Variable: *Type 2 Diabetes Mellitus*

Type 2 diabetes mellitus was considered a mediator variable in the analysis. Information on this variable was obtained from the administrative databases, where the follow-up years extended from 1989 to 2014. There were five versions of the Diabetes Canada clinical practice guidelines published in this time frame (in 1992, 1998, 2003, 2008, and 2013); however, the diagnostic criteria remained relatively consistent between these versions.

For the purposes of this study, a woman was coded as having this condition if she had either of two or more physician visits within two years, one hospital visit, or cause of death with diagnosis codes pertaining to T2DM. The date of diagnosis was the date of first contact for T2DM. A study assessing the validity of administrative data for ascertaining cases of diabetes found this algorithm was valid as it was associated with a sensitivity of 81.9% and a specificity of 99.2% (154).

The specific codes pertaining to T2DM were ICD-9 250.x0 or 250.x2 and ICD-10-CA E11. Because no decimals were included in the ICD-9 diagnosis codes between 1989 and 1997, the code 250 was used, which does not allow distinction between T1DM and T2DM. With the exclusion of women with pre-existing diabetes based on information recorded in the NSAPD, almost all diabetes cases that arose in the population of interest would be T2DM. As previously mentioned, women who were diagnosed with T2DM up to six months after their delivery was recorded as having unrecognized pre-existing diabetes and were excluded from the study.

4.5.3 Outcome Variable: *Cardiovascular Disease*

In this study, the primary outcome was a composite of CVD, defined as IHD, PAD, stroke of any type including TIAs, percutaneous coronary intervention, coronary artery bypass graft, or carotid endarterectomy. This information was derived from the physician billing, hospital discharge databases, and vital statistics. A woman was coded as having CVD as an outcome if she had either two physician visits within two years, one hospital visit, or cause of death with the diagnosis or procedure codes pertaining to CVD. The date of diagnosis was the earliest of visits that comply with this definition.

The specific ICD-9, ICD-10-CA, and Canadian Classification of Interventions codes that were used to identify the outcome of CVD are listed in Table 4-1. Ischaemic heart disease and stroke were also examined separately as part of the analysis for the secondary objective.

Tu et al. (155) found that the combination of physician billings and hospital discharge abstracts is a valid algorithm to identify patients with IHD. Two physician billings within a one-year period or a hospital discharge abstract yielded a sensitivity of 77.0% (95% CI: 68.2%-85.9%), a specificity of 98.0% (95% CI: 97.0%-98.9%), and a positive predictive value of 78.8% (95% CI: 70.1%-87.5%) (155). Likewise, Kokotailo et al. (156) determined that passive surveillance of stroke using ICD-9 and ICD-10 codes are useful in identifying true cases of stroke: ICD-9 codes identified stroke cases with a sensitivity of 90% (95% CI: 86%-93%) and ICD-10 codes identified stroke cases with a sensitivity of 92% (95% CI: 88%-95%). The difference in sensitivity between ICD-9 and ICD-10 codes was not statistically significant (p=0.865). A study of the reliability of hospital administrative data (using ICD-10 codes) for identifying stroke in Ontario corroborated these results and found that the positive predictive value of inpatient hospital administrative data was 89.5% (95% CI: 88.0-91.0) for TIA, 91.9%

(95% CI: 90.2-93.5) for intracerebral hemorrhage, and 97.3% (95% CI: 96.9-97.7) for ischaemic stroke and that the positive predictive value of emergency department administrative data was 95.3% (95% CI: 94.6–96.0) for TIA, 86.3% (95% CI: 76.8–95.7) for intracerebral hemorrhage, and 78.8% (95% CI: 76.3–81.2) for ischaemic stroke (157).

4.5.4 Confounders

Selected demographic and maternal characteristics recorded in the NSAPD were included as potential confounders. These variables were chosen a priori because they are known to be associated with both GDM and CVD. These variables include cigarette smoking, maternal age at first pregnancy, pre-pregnancy weight, area of residence (urban or rural, based on the Canadian postal code), area-level income quintile derived from Census of Canada information, parity, pre-existing hypertension in the first pregnancy, and marital status.

Smoking in any pregnancy is recorded in the NSAPD as the number of cigarettes smoked per day before the mother became pregnant, at the time of the first prenatal visit, at the time of prenatal visit from 18-22 weeks, and at the time of delivery. For the purposes of this study, smoking status in any pregnancy was used and was a binary variable: ever smoked and never smoked.

Maternal age at first pregnancy was derived by subtracting the mother's birth year from the year of first delivery. It was used as a continuous variable in this study.

Maternal pre-pregnancy weight is recorded on the Prenatal Record or the Maternal Admission Assessment and represents the mother's weight before pregnancy or at the first prenatal visit (weight gain in the first trimester is low and, therefore, close to pre-pregnancy weight (158)). It is either measured by a health care provider or is self-reported by the woman. For the purposes of

this study, maternal pre-pregnancy weight at the last recorded pregnancy was used and was a continuous variable.

Rural residence was derived using a woman's postal code, which is recorded on the Hospital Admission Form. It was determined that the woman resided in a rural area if the second position in the postal code is a "0" and that she resided in an urban area if the second position in the postal code is any digit other than "0". Rural residence at the last pregnancy was used and was a binary variable.

Area-level income quintile (Quintile of annual income per person equivalent (QAIPPE)) was also derived using a woman's postal code. Statistics Canada releases QAIPPE, an area-based socioeconomic measure of neighbourhood income per person equivalent, as a component of the census program (159). To derive QAIPPE, Statistics Canada calculates the average income per person for each dissemination area (a small geographic area composed of one or more neighbouring blocks, with a population of 400 to 700 persons) (160). Twenty percent of dissemination areas with the lowest incomes have a QAIPPE of "1" and 20% of the dissemination areas with the highest incomes have a QAIPPE of "5" (159). The woman's postal code, as recorded on the Hospital Admission Form, was linked to these income quintiles through the Postal Code Conversion File (161). For the purposes of this study, the QAIPPE at the last recorded pregnancy was used as a categorical variable.

Parity is defined as the number of pregnancies that resulted in one or more infants weighing 500 g or more at birth or 20 weeks or greater gestational age (regardless of whether the infants lived, were stillborn, or died after birth). This information is recorded on the Prenatal Record, Maternal Admission Assessment, or Physician's Assessment. Parity was a binary variable in this study: gave birth one time or gave birth two or more times.

Pre-existing hypertension in the first pregnancy is recorded on the Prenatal Record or Discharge Summary and was a binary variable: yes or no.

Marital status is recorded on the Hospital Admission Form or Prenatal Record and was coded as either single, married, widowed, divorced, separated, common-law, or unknown. Marital status was a binary variable in this study: married and common-law was one level and all other marital statuses was another level.

4.6 Statistical Analysis

4.6.1 Data Management

Requested data were obtained for the parent study from the RCP and HDNS. Data were stored on an HDNS computer in a directory accessible only to authorized members of the research team. Data were cleaned, which involved reformatting values and recoding variables. Variables were also inspected to determine whether the values or codes were reasonable. The data were analyzed using the statistical software packages Stata (StataCorp. 2015. *Stata Statistical Software: Release 14.* College Station, TX: StataCorp LP) and R (R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) with the packages *survival*, *survreg*, and *Amelia*.

4.6.2 Descriptive Statistics

A descriptive analysis of baseline characteristics, or potential confounders, was conducted in the first step and these characteristics included smoking during pregnancy, maternal age at first delivery, maternal pre-pregnancy weight, rural residence, QAIPPE, parity, pre-existing hypertension in the first pregnancy, and marital status. A measure of central tendency

(mean) and variability (standard deviation) were calculated for continuous variables, along with proportions for categorical variables. This analysis was conducted for the entire group and then by GDM status. For the latter, t-tests and Chi-squared or Fisher's exact tests were performed to assess the association of these variables with GDM, the exposure of interest. The number of women who developed T2DM and CVD was calculated by covariate status as well as by GDM status, along with the incidence rate (per 10,000 person-years).

An exploratory analysis was then conducted by estimating uHR to assess whether these variables were associated with the potential mediator, T2DM, and the outcome of interest, CVD. Correlations among confounders were examined to identify collinearity.

4.6.3 Unadjusted and Multiple Regression

This study consisted of time-to-event data and, therefore, survival analysis was conducted. The follow-up period began at the last recorded pregnancy for each woman. A woman was censored when the outcome of interest, death, migration from the province, or the end of the study period occurred. For this analysis, a woman was placed in the GDM group if she had been diagnosed with GDM in any of her pregnancies. Likewise, a woman was placed in the no GDM group if she had never been diagnosed with GDM in any of her pregnancies.

The first step in this analysis involved calculating Kaplan-Meier survival curves separately for the GDM and no GDM groups and separately for the outcomes T2DM and CVD. Survival times were compared between the groups by conducting a log-rank test, where the null hypothesis was that there was no difference regarding survival among the GDM and no GDM groups.

The analysis of primary objective I (quantifying the association between GDM and CVD) then involved a univariable analysis using a simple Cox proportional hazards regression model with GDM as the explanatory variable, as shown below:

$$h(t) = h_0(t)\exp(b_1X_1) \tag{1}$$

where $h(t)$ = the expected hazard at time t

$h_0(t)$ = the baseline hazard and represents the hazard when X_1 is equal to zero

X_1 = GDM

Before proceeding with Cox proportional hazards regression, the proportional hazards assumption was checked by conducting a graphical analysis (i.e., -ln-ln curve) and a goodness-of-fit test for all variables. An uHR was estimated with a 95% CI in this step using GDM as the sole explanatory variable (X_1) and CVD as the outcome.

Multivariable analysis was then conducted using a Cox proportional hazards regression model with a combination of explanatory variables, which can be expressed as:

$$h(t) = h_0(t)\exp(b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_pX_p) \tag{2}$$

where $h(t)$ = the expected hazard at time t

$h_0(t)$ = the baseline hazard and represents the hazard when X_i is equal to zero.

The explanatory variables in this model included GDM and the potential confounders outlined previously. Variables that violated the proportional hazards assumption (only parity) were put into strata in the final, fully adjusted models. An aHR and the 95% CI was obtained from this model.

These statistical procedures were repeated for the outcomes of stroke and IHD separately to meet the secondary objective.

As preliminary analyses for primary objective II (examining mediation of the association between GDM and CVD by T2DM), these steps were repeated, except with T2DM as the outcome instead of CVD.

The main analyses were complete case analyses using the listwise deletion procedure. Multiple imputation using chained equations was used also to replace missing values of covariates in 50 imputed datasets. Because both methods yielded similar aHRs for the associations between GDM and CVD, GDM and stroke, and GDM and IHD, only the results from the complete case analysis are presented.

4.6.4 Mediation Analysis

Mediation analysis was used to address primary objective II (examining mediation of the association between GDM and CVD by T2DM). As outlined in section 2.4.3, the purpose of mediation analysis is to decompose the total effect of the association between GDM and CVD into the indirect effect and direct effect (Figures 2-1, 2-2, and 2-3).

Two previous studies evaluated the single and joint associations of GDM and T2DM on the risk of developing CVD later in life as a way of exploring mediation (12,143). Joint analyses were also examined in this study, so comparisons could be made to the previous studies. To do this, women with and without exposure to GDM were further categorized by progression to T2DM and four groups were created: no GDM and no progression to T2DM, GDM and no progression to T2DM, no GDM and progression to T2DM, and GDM and progression to T2DM. Adjusted hazard ratios were obtained from these Cox regression models and were compared.

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The procedure proposed by Lange and colleagues (162,163) addresses some of the limitations that exist in the traditional approach to mediation analysis. The new method is based on counterfactuals and marginal structural models that directly parameterize the natural direct and indirect effects of interest. This new method estimates the parameters of the marginal structural model using inverse probability of treatment weights, which creates a pseudo-population that removes covariate imbalances between exposure groups, making the estimation of causal effects more accurate (162-164).

Although the procedure by Lange et al. (162,163) can be implemented in the open source statistical software package R with a user-written program, *medflex* (165), the latter program is not yet capable of handling survival data. Lange and colleagues, (162) therefore, developed a series of steps and code for estimating the direct, indirect, and total effects in R, which was adapted for this study and involved: 1) fitting a parametric Weibull survival model to the CVD survival times using GDM status, T2DM status, and confounders, 2) imputing nested counterfactuals, 3) fitting a Cox model including the observed GDM status, the counterfactual GDM status, and all confounders, and 4) establishing CIs by 1,000 bootstrap replications of the previous steps. The output in R includes HRs for the indirect, direct, and total effects, as well as an estimate of the mediated proportion. Using this formal mediation method likely improved the internal validity of my study as it can provide better control for confounders and selection bias than traditional methods by improving adjustment for group differences (164).

4.7 Sample Size and Power Calculation

The minimally-detectable RR for the association between GDM and CVD given the sample size available for this study was calculated a priori. The number of women in this cohort

who had data linked in both the NSAPD from their first pregnancy and the administrative databases was estimated to be 94,000 mothers. Approximately 0.5% of these women were projected to be excluded due to pre-existing diabetes, leaving a total cohort of approximately 93,000 mothers. The estimated cumulative incidences of GDM and CVD were approximately 4% and 1%, respectively, in the cohort. With this sample size, prevalence of exposure, incidence of CVD, a power of 80%, and an alpha of 0.05, the minimally-detectable RR, calculated using Stata, was 1.5.

Table 4-1. Selected ICD-9 and ICD-10-CA codes used to define cardiovascular disease

	ICD-9	ICD-10-CA
Ischaemic Heart Disease		
Angina pectoris	413	I20
Acute myocardial infarction	410	I21
Subsequent myocardial infarction	N/A	I22
Other acute ischaemic heart diseases	411	I24
Chronic ischaemic heart disease	414	I25
Old myocardial infarction	412	N/A
Cerebrovascular Disease		
Cerebral infarction	N/A	I63
Haemorrhagic stroke	430, 431, 432	I60, I61, I62
Stroke, not specified as haemorrhage or infarction	N/A	I64
Occlusion and stenosis of precerebral arteries	433	I65
Occlusion and stenosis of cerebral arteries	434	I66
Transient cerebral ischaemia	435	G45 (excluding G45.4)
Diseases of Arteries, Arterioles, and Capillaries		
Peripheral artery disease / atherosclerosis	440	I70
Intervention	**ICD-9**	**Canadian Classification of Interventions**
Coronary Artery Bypass Graft	36.1	1.IJ.76
Coronary angioplasty or endarterectomy	36.0 or 38.1	1.IJ.50, 1.IJ.55, 1.IJ.57
Carotid angioplasty or endarterectomy		1.JE.57, 1.JE.50

Chapter 5 RESULTS

5.1 Characteristics of the Cohort

A total of 90,183 Nova Scotian women had a first birth between 1988 and 2012. After excluding 2551 women with missing information on date of birth or who had pre-existing T1DM, T2DM, and CVD, 87,632 women were included in the study (Figure 5-1; all figures and tables can be found at the end of the chapter). Of the 87,632 women included in the study, 3549 (4.0%) developed GDM in at least one pregnancy and 1224 (1.4%) developed CVD. Of the 3549 women who developed GDM in at least one pregnancy, 89 (2.5%) developed CVD.

A detailed description of the characteristics of the study cohort is provided in Table 5-1. The frequencies and means of covariates obtained from the NSAPD are displayed in this table, along with the percentage of missing values. The mean (standard deviation (SD)) of mother's age at first pregnancy was 26.3 years (5.5 years) and the mean (SD) for pre-pregnancy weight was 68.8 kg (16.6 kg).

5.2 Characteristics of the Cohort by Gestational Diabetes Mellitus Status

The associations between GDM and covariates obtained from the NSAPD are shown in Table 5-1. All covariates except for smoking during pregnancy were statistically significantly different between women who developed GDM in at least one pregnancy and women who did not develop GDM in any pregnancies. Covariates that were associated with a history of GDM included age at first birth, pre-pregnancy weight, pre-existing hypertension, rural residence, area-level income, parity, and marital status.

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5.3 PRIMARY OBJECTIVE I: Quantify the risk of subsequent cardiovascular disease associated with gestational diabetes mellitus

The first step of the analysis of primary objective I involved examining the associations between the covariates listed in Table 5-1 and CVD to give an indication about the potential for confounding between GDM and CVD. The association between GDM and CVD was then evaluated by fitting unadjusted and adjusted Cox regression models.

5.3.1 Association between Covariates and Cardiovascular Disease

The number of women who developed CVD in each covariate category, incidence rates (per 10,000 person-years), and uHRs for each covariate's association with CVD are shown in Table 5-2. Smoking during pregnancy, age at first pregnancy, pre-pregnancy weight, and pre-existing hypertension, were all positively associated with CVD, while living in an urban area was inversely associated with CVD. No association was observed between CVD and giving birth two or more times, area-level income quintile, and marital status.

5.3.2 Association between Gestational Diabetes Mellitus and Cardiovascular Disease

A total of 1224 women developed CVD within a median follow-up period of 11.8 years. Incidence rates (per 10,000 person-years) of developing CVD for women with and without GDM in addition to uHRs and aHRs are shown in Table 5-3. The number of women with a history of GDM who developed CVD was 89. The associated incidence rate was 26.0 per 10,000 person-years for women with a history of GDM, compared to 13.2 per 10,000 person-years for women without a history of GDM.

In the unadjusted Cox regression model, women with a history of GDM had an estimated 105% higher risk of developing CVD later in life compared to women without GDM [uHR=2.05

(95% CI: 1.65-2.54)]. Adjustment for confounding variables attenuated the relationship between GDM and subsequent CVD; however, women with a history of GDM still had an estimated 53% higher risk of developing CVD later in life compared to women without GDM in the adjusted model [aHR=1.53 (95% CI: 1.23-1.91)]. Pre-pregnancy weight was the covariate that was responsible for most of the attenuation: Removing it from the model induced a 15% change in the HR for the association between GDM and CVD (from 1.53 to 1.76).

5.4. PRIMARY OBJECTIVE II: Estimate the extent to which T2DM acts as a mediator of association between GDM and CVD

Like the analysis of primary objective I, the analysis of primary objective II began by assessing the association of the covariates with T2DM to give an indication of the potential for confounding. The association between GDM and T2DM was then assessed by fitting Cox regression models. Joint associations of GDM and T2DM on the risk of developing CVD were then evaluated for the purpose of comparison with the literature. Finally, a formal mediation analysis was conducted using a modified version of the approach proposed by Lange and colleagues (162).

5.4.1 Association between Covariates and Type 2 Diabetes Mellitus

The number of women who developed T2DM in each covariate category, the incidence rate (per 10,000 person-years), and uHRs are shown in Table 5-4. All covariates were associated with T2DM except the fourth area-level income quintile and parity. An inverse relationship was observed between T2DM and living in an urban area and being in the uppermost area-level income quintile.

5.4.2 Association between Gestational Diabetes Mellitus and Type 2 Diabetes Mellitus

Incidence rates (per 10,000 person-years) of developing T2DM in women with and without GDM in addition to uHRs and aHRs are shown in Table 5-5. The number of women with a history of GDM who developed T2DM was 685. The associated incidence rate was 230.4 per 10,000 person-years for women with a history of GDM, compared to 20.2 per 10,000 person-years for women without a history of GDM. In the unadjusted Cox regression model, the risk of developing T2DM was 12 times higher among women with a history of GDM than women without a history of GDM [uHR=12.2 (95% CI: 11.2-13.3)]. After adjustment for the covariates, the risk of developing T2DM was 7.99 times greater among women with a history of GDM than women without a history of GDM [aHR=7.99 (95% CI: 7.28-8.76)] (Table 5-5).

5.4.3 Association between Gestational Diabetes Mellitus and Cardiovascular Disease by Progression to Type 2 Diabetes Mellitus

Women with exposure to GDM were further categorized by progression to T2DM to assess whether the relationship between GDM and CVD can be explained by progression to T2DM (Table 5-6). The incidence rates of CVD per 10,000 person-years were 12.4 in the no GDM or T2DM group, 16.5 in the GDM, no T2DM group, 87.8 in the T2DM, no GDM group, and 88.6 in the GDM and T2DM group. Compared with the reference group of women without a history of GDM or T2DM, women who developed T2DM but did not have a history of GDM and women who had a history of GDM and developed T2DM had three times greater risk of developing CVD in the fully adjusted models [aHR=3.19 (95% CI: 2.52-4.04); aHR=3.64 (95% CI: 2.62-5.06), respectively]. Women who had a history of GDM but did not develop T2DM did not have an elevated risk of subsequent CVD [aHR=1.16 (95% CI: 0.87-1.55)]. The interaction between GDM and T2DM was not significant.

5.4.4 Mediation of the Association between Gestational Diabetes Mellitus and Cardiovascular Disease by Type 2 Diabetes Mellitus

Mediation analysis was performed to estimate the extent to which the association between GDM and CVD could be explained by progression to T2DM (Table 5-7). After controlling for confounders and stratifying by parity, GDM was associated with an increase in risk of developing CVD of 62% [aHR=1.62 (95% CI: 1.50-1.75)]. The effect of GDM on CVD has, theoretically, two components: the indirect effect through progression to T2DM, which increased the risk of developing CVD by 58% [aHR=1.58 (95% CI: 1.46-1.70)], and the effect through all other pathways, which was not significant. The proportion of the association between GDM and CVD that was mediated through T2DM was 93.9% (95% CI: 86.8%-101.8%) (Table 5-7). This indicates that the estimated effect between GDM and CVD is nearly fully mediated by T2DM.

5.5 SECONDARY OBJECTIVE: Explore primary objectives I and II by type of Cardiovascular Disease

5.5.1 Gestational Diabetes Mellitus and Stroke

A total of 253 women had a stroke during the study period. The number of women who developed stroke in each covariate category, the incidence rate (per 10,000 years), and uHRs for the association between each covariate and risk of stroke are shown in Table 5-8. Smoking during pregnancy was the only covariate significantly associated with stroke and this association was positive.

Incidence rates (per 10,000 years) of developing stroke for women with and without GDM in addition to uHRs and aHRs are shown in Table 5-9. Of the women with a history of GDM, 12 developed stroke, while 241 women without a history of GDM developed stroke. The

associated incidence rate was 3.5 per 10,000 person-years for women with a history of GDM, compared to 2.8 per 10,000 person-years for women without a history of GDM. In the unadjusted Cox regression model, GDM was not associated with the risk of stroke [uHR=1.29 (95% CI: 0.72-2.31)]. After adjustment for covariates, the association remained nonsignificant [aHR=1.19 (95% CI: 0.66-2.15)] (Table 5-9).

Because no total association was found between GDM and stroke in this study and because the associations between GDM and T2DM and T2DM and stroke were found to be positive in previous studies (8,10), mediation of the association between GDM and stroke by T2DM (Primary objective II) was not examined.

5.5.2 Gestational Diabetes Mellitus and Ischaemic Heart Disease

A total of 978 women developed IHD during the study period. The number of women who developed IHD in each covariate category, along with the incidence rate (per 10,000 years), and uHRs for the association between covariates and IHD risk are shown in Table 5-10. Having pre-existing hypertension in the first pregnancy, smoking during pregnancy, giving birth two or more times, and having T2DM were positively and significantly associated with IHD.

Incidence rates (per 10,000 years) of developing IHD in women with and without GDM in addition to uHRs and aHRs are shown in Table 5-11. The number of women with a history of GDM who developed IHD was 78, while the number of women without a history of GDM who developed IHD was 900. The associated incidence rate was 22.8 per 10,000 person-years for women with a history of GDM, compared to 10.4 per 10,000 person-years for women without a history of GDM. Women with a history of GDM experienced an estimated 127% higher risk of developing IHD later in life compared to women without GDM [uHR=2.27 (95% CI: 1.80-

2.86)]. GDM remained positively associated with IHD risk after adjusting for the covariates

listed in Table 5-1 and after stratifying by parity [uHR=1.61 (95% CI: 1.27-2.04)].

In examining the joint effects of GDM and T2DM, the incidence rates of IHD per 10,000

person-years were 9.8 in the no GDM or T2DM group, 13.8 in the GDM, no T2DM group, 73.0

in the T2DM, no GDM group, and 82.0 in the GDM and T2DM group (Table 5-12). A similar

pattern was seen with IHD events compared to CVD events, with an elevated risk of IHD in

women who developed T2DM but did not have a history of GDM and women who had a history

of GDM and developed T2DM in the fully adjusted models when compared with the reference

group of women without a history of GDM or T2DM [aHR=3.14 (95% CI: 2.42-4.07);

aHR=3.91 (95% CI: 2.77-5.51), respectively]. Women who had a history of GDM but did not

develop T2DM did not experience an elevated risk of subsequent IHD [aHR=1.19 (95% CI:

0.87-1.63)], which is similar to what was seen when CVD was the outcome.

Results of the mediation analysis with IHD as the outcome and T2DM as the mediator

are shown in Table 5-13. After controlling for confounders and stratifying by parity, GDM was

associated with an increase in risk of developing IHD of 70% [aHR=1.70 (95% CI: 1.57-1.85)].

Women with a history of GDM experienced an estimated 7% elevated risk of developing IHD

later in life compared to women without GDM, independent of T2DM [direct effect: aHR=1.07

(95% CI: 1.02-1.11)]. The proportion of the association between GDM and IHD that was

mediated through T2DM was 87.9% (95% CI: 80.0%-95.9%) and, therefore, the estimated effect

between GDM and IHD was nearly fully mediated by T2DM.
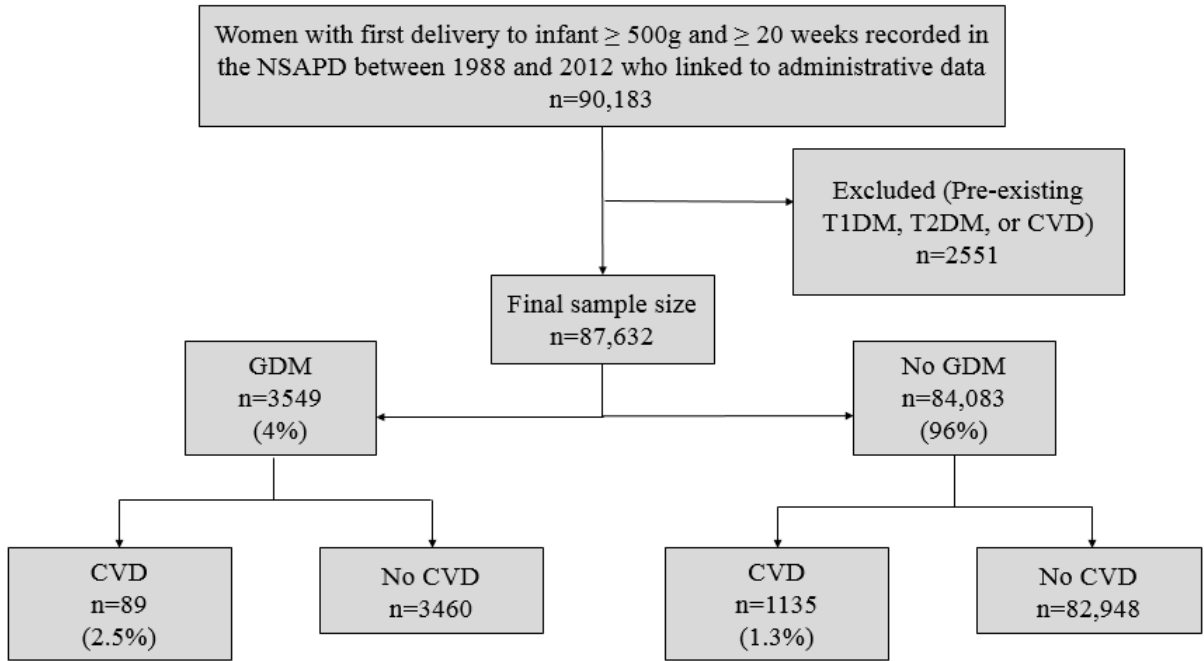
Figure 5-1. Flow diagram of women included in the study

Table 5-1. Characteristics of the cohort, overall and by GDM status

Characteristic	N or mean	(% or SD)	No GDM N=84,083		GDM N=3549		p-value[a]
			N or mean	(Row % or SD)	N or mean	(Row % or SD)	
Age at first pregnancy (years)	26.3	(5.5)	26.3	(5.5)	27.3	(5.5)	0.001
Pre-pregnancy weight[b] (kg)	68.8	(16.6)	68.4	(16.3)	79.4	(21.0)	0.001
Pre-existing hypertension in first pregnancy							0.001
No	86,745	(99.0)	83,300	(96.0)	3445	(4.0)	
Yes	887	(1.0)	783	(88.3)	104	(11.7)	
Smoking in any pregnancy							0.129
No	63,371	(72.3)	60,839	(96.0)	2532	(4.0)	
Yes	23,557	(26.9)	22,562	(95.8)	995	(4.2)	
Missing	704		682		22		
Urban residence[b]							0.001
No	31,080	(35.5)	29,681	(95.5)	1399	(4.5)	
Yes	49,422	(56.4)	47,703	(96.5)	1719	(3.5)	
Missing	7130		6699		431		
Area-level income quintile[b]							0.001
1	17,020	(19.4)	16,245	(95.5)	775	(4.6)	
2	18,653	(21.3)	17,915	(96.0)	738	(4.0)	
3	19,931	(22.7)	19,097	(95.8)	834	(4.2)	
4	18,088	(20.6)	17,386	(96.1)	702	(3.9)	
5	13,551	(15.5)	13,065	(96.4)	486	(3.6)	
Missing	389		375		14		
Parity[b]							0.001
1	35,430	(40.4)	34,355	(97.0)	1075	(3.0)	
≥ 2	52,202	(60.0)	49,728	(95.3)	2474	(4.7)	
Marital status[b]							0.001
Married/common-law	22,451	(25.6)	21,671	(96.5)	780	(3.5)	
Single/widowed/divorced	62,077	(70.8)	59,416	(95.7)	2661	(4.3)	
Missing	3104	(3.5)	2996		108		

[a] p-value based on Chi-square test (categorical variables) or t-test (continuous variable) comparing the GDM group to the no GDM group

[b] Based on information at the last pregnancy

Table 5-2. Association between covariates and the risk of cardiovascular disease[a]

Characteristic	CVD (N)	Person-years	Incidence rate (per 10,000 person-years)	uHR (95% CI)
Age at first pregnancy (per 5 years)	—[b]	—[b]	—[b]	1.31 (1.24-1.38)
Pre-pregnancy weight (per 5 kg)	—[b]	—[b]	—[b]	1.09 (1.07-1.10)
Pre-existing hypertension in first pregnancy				
No	1188	886,889	13.4	1.00 (Ref)
Yes	36	8652	41.6	3.07 (2.21-4.28)
Smoking in any pregnancy				
No	795	646,072	12.3	1.00 (Ref)
Yes	429	249,469	17.2	1.32 (1.18-1.49)
Urban residence[c]				
No	562	369,169	15.2	1.00 (Ref)
Yes	662	526,373	12.6	0.88 (0.79-0.99)
Area-level income quintile[c]				
1	243	170,418	14.3	1.03 (0.87-1.22)
2	273	191,999	14.2	1.02 (0.86-1.20)
3	294	211,399	13.9	1.00 (Ref)
4	238	182,849	13.0	0.95 (0.80-1.13)
5	176	138,877	12.7	0.92 (0.76-1.11)
Parity[b]				
1	410	309,938	13.2	1.00 (Ref)
≥ 2	814	585,604	13.9	1.11 (0.99-1.26)
Marital status[b]				
Married/common-law	981	699,141	14.0	1.00 (Ref)
Single/widowed/divorced	243	196,400	12.4	0.94 (0.82-1.09)

CI, confidence interval; CVD, cardiovascular disease; uHR, unadjusted hazard ratio
[a] Among women without missing values
[b] Not shown for continuous covariates
[c] Based on information at the last pregnancy

Table 5-3. Association between gestational diabetes mellitus and the risk of cardiovascular disease

	No GDM	GDM
Person-years	861,303	34,239
CVD (N[a])	1135	89
CVD rate (per 10,000 person-years)	13.2	26.0
uHR (95% CI)	1.00 (Ref)	2.05 (1.65-2.54)
aHR[b] (95% CI)	1.00 (Ref)	1.53 (1.23-1.91)

aHR, adjusted hazard ratio; CI, confidence interval; CVD, cardiovascular disease; GDM, gestational diabetes mellitus; uHR, unadjusted hazard ratio

[a] Among women with no missing values in confounding variables

[b] adjusted for age at first pregnancy (per 5 years), pre-existing hypertension in first pregnancy, smoking in any pregnancy, pre-pregnancy weight (per 5 kg), urban residence, area-level income quintile, parity, marital status and stratified by parity

Table 5-4. Association between covariates and the risk of type 2 diabetes mellitus

Characteristic	T2DM (Nᵃ)	Person-years	Incidence rate (per 10,000 person-years)	uHR (95% CI)
Age at first pregnancy (per 5 years)	—[b]	—[b]	—[b]	1.10 (1.06-1.14)
Pre-pregnancy weight (per 5 kg)	—[b]	—[b]	—[b]	1.24 (1.23-1.26)
Pre-existing hypertension in first pregnancy				
No	2299	873,638	26.3	1.00 (Ref)
Yes	103	7940	129.7	4.99 (4.10-6.08)
Smoking in any pregnancy				
No	1606	636,928	25.2	1.00 (Ref)
Yes	796	244,650	32.5	1.24 (1.14-1.35)
Urban residence[c]				
No	1061	362,836	29.2	1.00 (Ref)
Yes	1341	518,742	25.9	0.93 (0.86-1.00)
Area-level income quintile[c]				
1	561	167,270	33.5	1.27 (1.13-1.43)
2	584	188,364	31.0	1.17 (1.04-1.31)
3	552	208,261	26.5	1.00 (Ref)
4	420	180,449	23.3	0.89 (0.78-1.01)
5	285	137,235	20.8	0.79 (0.68-0.91)
Parity[c]				
1	812	304,844	26.6	1.00 (Ref)
≥ 2	1590	576,735	27.6	1.05 (0.97-1.15)
Marital status[c]				
Married/common-law	1835	688,313	26.7	1.00 (Ref)
Single/widowed/divorced	567	193,265	29.3	1.16 (1.06-1.27)

CI, confidence interval; T2DM, type 2 diabetes mellitus; uHR, unadjusted hazard ratio
[a] Does not equal the total sample size due to missing values
[b] Not shown for continuous covariates
[c] Based on information at the last pregnancy

Table 5-5. Association between gestational diabetes mellitus and the risk of type 2 diabetes mellitus

	No GDM	GDM
Person-years	851,853	29,725
T2DM (N[a])	1717	685
T2DM rate (per 10,000 person-years)	20.2	230.4
uHR (95% CI)	1.00 (Ref)	12.2 (11.2-13.3)
aHR[b] (95% CI)	1.00 (Ref)	7.99 (7.28-8.76)

aHR, adjusted hazard ratio; CI, confidence interval; CVD, cardiovascular disease; GDM, gestational diabetes mellitus; T2DM, type 2 diabetes mellitus; uHR, unadjusted hazard ratio
[a] Does not equal the total sample size due to missing values
[b] adjusted for age at first pregnancy (per 5 years), pre-existing hypertension in first pregnancy, smoking in any pregnancy, pre-pregnancy weight (per 5 kg), urban residence, area-level income quintile, parity, marital status and stratified by parity

Table 5-6. History of gestational diabetes mellitus by progression to type 2 diabetes mellitus and risk of future cardiovascular disease

	No GDM or T2DM	GDM only	T2DM only	GDM and T2DM
Total CVD				
Person-years	851,853	29,725	9449	4514
CVD (N[a])	1052	49	83	40
CVD rate (per 10,000 person-years)	12.4	16.5	87.8	88.6
uHR (95% CI)	1.00 (Ref)	1.44 (1.08-1.92)	4.28 (3.41-5.38)	5.52 (4.02-7.57)
aHR[b] (95% CI)	1.00 (Ref)	1.16 (0.87-1.55)	3.19 (2.52-4.04)	3.64 (2.62-5.06)

aHR, adjusted hazard ratio; CI, confidence interval; CVD, cardiovascular disease; GDM, gestational diabetes mellitus; T2DM, type 2 diabetes mellitus; uHR, unadjusted hazard ratio

[a] Does not equal the total sample size due to missing values

[b] adjusted for age at first pregnancy (per 5 years), pre-existing hypertension in first pregnancy, smoking in any pregnancy, pre-pregnancy weight (per 5 kg), urban residence, area-level income quintile, parity, marital status and stratified by parity

Table 5-7. Mediation of the association between gestational diabetes mellitus and cardiovascular disease by type 2 diabetes

Effect	Interpretation	HR	(95% CI)
Natural indirect	Effect of GDM on CVD explained by T2DM	1.58	(1.46-1.70)
Natural direct	Effect of GDM on CVD unexplained by T2DM	1.03	(0.99-1.07)
Total	Sum of direct effect and indirect effect	1.62	(1.50-1.75)
% Mediated		93.9	(86.8-101.8)

CI, confidence interval; CVD, cardiovascular disease; GDM, gestational diabetes mellitus; HR, hazard ratio; T2DM, type 2 diabetes mellitus

Table 5-8. Association between covariates and risk of stroke

Characteristic	Stroke (N[a])	Person-years	Incidence rate (per 10,000 person-years)	uHR (95% CI)
Age at first pregnancy (per 5 years)	— [b]	— [b]	— [b]	1.04 (0.92-1.17)
Pre-pregnancy weight (per 5 kg)	— [b]	— [b]	— [b]	1.03 (0.99-1.07)
Smoking during pregnancy				
No	163	646,072	2.5	1.00 (Ref)
Yes	90	249,469	3.6	1.37 (1.06-1.77)
Urban residence[c]				
No	117	369,169	3.2	1.00 (Ref)
Yes	136	526,373	2.6	0.86 (0.67-1.10)
Area-level income quintile[c]				
1	51	170,418	3.0	1.11 (0.76-1.62)
2	55	191,999	2.9	1.06 (0.73-1.53)
3	57	211,399	2.7	1.00 (Ref)
4	46	182,849	2.5	0.94 (0.64-1.39)
5	44	138,877	3.2	1.18 (0.80-1.75)
Parity[c]				
1	95	309,938	3.1	1.00 (Ref)
≥ 2	158	585,604	2.7	0.93 (0.71-1.20)
Marital status[c]				
Married/common-law	197	699,141	2.8	1.00 (Ref)
Single/widowed/divorced	56	196,400	2.9	1.07 (0.79-1.44)

CI, confidence interval; uHR, unadjusted hazard ratio
[a] Does not equal the total sample size due to missing values
[b] Not shown for continuous covariates
[c] Based on information at the last pregnancy

Table 5-9. Association between gestational diabetes mellitus and risk of stroke

	No GDM	GDM
Person-years	861,303	34,239
Stroke (N[a])	241	12
Stroke rate (per 10,000 person-years)	2.8	3.5
uHR (95% CI)	1.00 (Ref)	1.29 (0.72-2.31)
aHR[b] (95% CI)	1.00 (Ref)	1.19 (0.66-2.15)

aHR, adjusted hazard ratio; CI, confidence interval; GDM, gestational diabetes mellitus; uHR, unadjusted hazard ratio

[a] Does not equal the total sample size due to missing values

[b] adjusted for age at first pregnancy (per 5 years), smoking in any pregnancy, pre-pregnancy weight (per 5 kg), urban residence, area-level income quintile, parity, marital status and stratified by parity

Table 5-10. Association between covariates and risk of ischaemic heart disease

Characteristic	IHD (N[a])	Person-years	Incidence rate (per 10,000 person-years)	uHR (95% CI)
Age at first pregnancy (per 5 years)	—[b]	—[b]	—[b]	1.39 (1.31-1.47)
Pre-pregnancy weight (per 5 kg)	—[b]	—[b]	—[b]	1.10 (1.08-1.12)
Pre-existing hypertension in first pregnancy				
No	944	886,889	10.6	1.00 (Ref)
Yes	34	8652	39.3	3.65 (2.59-5.14)
Smoking during pregnancy				
No	639	646,072	9.9	1.00 (Ref)
Yes	339	249,469	13.6	1.30 (1.14-1.48)
Urban residence[c]				
No	446	369,169	12.1	1.00 (Ref)
Yes	532	526,373	10.1	0.90 (0.79-1.02)
Area-level income quintile[c]				
1	192	170,418	11.3	1.01 (0.83-1.22)
2	218	191,999	11.4	1.01 (0.84-1.21)
3	237	211,399	11.2	1.00 (Ref)
4	197	182,849	10.8	0.98 (0.81-1.18)
5	134	138,877	9.6	0.87 (0.70-1.07)
Parity[c]				
1	317	309,938	10.2	1.00 (Ref)
≥ 2	661	585,604	11.3	1.17 (1.02-1.34)
Marital status[c]				
Married/common-law	790	699,141	11.3	1.00 (Ref)
Single/widowed/divorced	188	196,400	9.6	0.91 (0.78-1.07)
T2DM				
No	872	881,578	9.9	1.00 (Ref)
Yes	106	13,964	75.9	4.91 (4.00-6.04)

CI, confidence interval; IHD, ischaemic heart disease; T2DM, type 2 diabetes mellitus; uHR, unadjusted hazard ratio

[a] Does not equal the total sample size due to missing values

[b] Not shown for continuous covariates

[c] Based on information at the last pregnancy

Table 5-11. Association between gestational diabetes mellitus and risk of ischaemic heart disease

	No GDM	GDM
Person-years	861,303	34,239
IHD (N[a])	900	78
IHD rate (per 10,000 person-years)	10.4	22.8
uHR (95% CI)	1.00 (Ref)	2.27 (1.80-2.86)
aHR[b] (95% CI)	1.00 (Ref)	1.61 (1.27-2.04)

aHR, adjusted hazard ratio; CI, confidence interval; GDM, gestational diabetes mellitus; IHD, ischaemic heart disease; uHR, unadjusted hazard ratio

[a] Does not equal the total sample size due to missing values

[b] adjusted for age at first pregnancy (per 5 years), pre-existing hypertension in first pregnancy, smoking in any pregnancy, pre-pregnancy weight (per 5 kg), urban residence, area-level income quintile, parity, marital status and stratified by parity

Table 5-12. History of gestational diabetes mellitus by progression to type 2 diabetes mellitus and risk of future ischaemic heart disease

	No GDM or T2DM	GDM only	T2DM only	GDM and T2DM
Total IHD				
Person-years	851,853	29,725	9449	4514
CVD (N[a])	831	41	69	37
CVD rate (per 10,000 person-years)	9.8	13.8	73.0	82.0
uHR (95% CI)	1.00 (Ref)	1.53 (1.12-2.10)	4.44 (3.45-5.71)	6.39 (4.59-8.90)
aHR[b] (95% CI)	1.00 (Ref)	1.19 (0.87-1.63)	3.14 (2.42-4.07)	3.91 (2.77-5.51)

aHR, adjusted hazard ratio; CI, confidence interval; GDM, gestational diabetes mellitus; IHD, ischaemic heart disease; T2DM, type 2 diabetes mellitus; uHR, unadjusted hazard ratio
[a] Does not equal the total sample size due to missing values
[b] adjusted for age at first pregnancy (per 5 years), pre-existing hypertension in first pregnancy, smoking in any pregnancy, pre-pregnancy weight (per 5 kg), urban residence, area-level income quintile, parity, marital status and stratified by parity

Table 5-13. Mediation of the association between gestational diabetes mellitus and ischaemic heart disease by type 2 diabetes

Effect	Interpretation	HR	(95% CI)
Natural indirect	Effect of GDM on IHD explained by T2DM	1.60	(1.47-1.74)
Natural direct	Effect of GDM on IHD unexplained by T2DM	1.07	(1.02-1.11)
Total	Sum of direct effect and indirect effect	1.70	(1.57-1.85)
% Mediated		87.9	(80.0-95.9)

CI, confidence interval; GDM, gestational diabetes mellitus; HR, hazard ratio; IHD, ischaemic heart disease; T2DM, type 2 diabetes mellitus

Chapter 6 DISCUSSION

6.1 Summary of Results

The results from this large retrospective study suggest that women with a history of GDM have a higher risk of developing CVD later in life compared to women without a history of GDM. Adjusting for many potential confounders including pre-pregnancy weight attenuated the relationship between GDM and future CVD; however, women with a history of GDM still had an estimated 53% (95% CI: 23%-91%) higher risk of developing CVD later in life compared to women without GDM. When examining the main types of CVD separately, GDM was associated with the risk of developing IHD, but was not associated with the risk of stroke.

The mediation analysis conducted in the present study suggests that the relationship between GDM and CVD is almost fully mediated by progression to T2DM [% mediated =93.9 (95% CI: 86.8-101.8)] and that the relationship between GDM and IHD is also almost fully mediated by progression to T2DM [% mediated =87.9 (95% CI: 80.0-95.9)].

6.2 Comparison with Previous Studies: Association between Gestational Diabetes Mellitus and Cardiovascular Disease

The results observed in the present study are consistent with what has been reported in previous studies of GDM and composite CVD end points (7,11,12,136,139-144,146). All these studies reported positive associations between GDM and CVD, although they differed in sample size, study population, follow-up time, and the confounders included in their statistical models (7,11,12,136,139-144,146).

The present study, to my knowledge, is one of the first large population-based cohort studies that has been able to adjust for pre-pregnancy body mass, which is a confounder of the relationship between GDM and CVD. Only four of the studies of the association between GDM and composite CVD end points adjusted for or stratified on body mass (139,142-144). Of these studies, one was a cross-sectional study with a small sample size (n=8127) (144), one was a case-control study with a small sample size (n=5949) (139), one was a population-based retrospective cohort study with half the sample size of the present study (n=47,909), and the other was a prospective cohort study with a large sample size (n=89,479), but was not population-based (143). The risk estimates decreased in each of these studies after the models were adjusted for body mass, and the risk estimates in the studies conducted by Tobias and colleagues (143) and Shostrom and colleagues (144) were lower than the risk estimates found in other studies that did not adjust for body mass (7,11,12,136). Together, these studies provide evidence that body mass is an important confounder that should be adjusted for in statistical models. Adjustment for pre-pregnancy weight attenuated the HR by 15% in the present study, which further highlights the importance of accounting for body mass.

The estimate of the association between GDM and CVD in the present study was still significant after adjustment for body mass. Results of all other studies that adjusted for body mass also found a significant association (139,142,143) except for one study (144). The exception was a recent cross-sectional study involving parous women from the 2007 to 2014 cycles of the National Health and Nutrition Examination Survey (NHANES) in the United States, which found a point estimate for the association between GDM and CVD similar to other studies but with a wide CI because the sample size in this study consisted of only 8127 women [aOR=1.52 (95% CI: 0.95-2.44)] (144). Additionally, the investigators based their outcome and

exposure assessment on self-reported history of GDM and CVD diagnoses, which may have led to misclassification bias. Information on BMI was collected at the time of the NHANES survey and not at the time of pregnancy and, therefore, adjustment for BMI several years postpartum may have resulted in residual confounding in this study and may have biased the RR estimate of the association between GDM and CVD towards the null.

The study conducted by Retnakaran and colleagues (12) was similar to the present study in that it was also a retrospective cohort study in a Canadian population and used a similar definition of CVD. They obtained a RR estimate that was slightly higher than the RR estimate obtained from the fully adjusted model in the present study [aHR = 1.78 (95% CI: 1.57-2.01)] and [aHR=1.53 (95% CI: 1.23-1.91)], respectively), with elevated risk of CVD later in life in women with a history of GDM compared to women without a history of GDM. Their estimate of risk may have been higher because they were unable to adjust for body mass and other important confounders, which largely attenuated the relationship between GDM and CVD in the present study. Kaul and colleagues (141) also conducted a study that was similar to the present study and yielded a comparable estimate of the RR. Their retrospective cohort study also involved a Canadian population and their definition of CVD included both IHD and stroke. The investigators also used a provincial perinatal database that was linked to health services utilization databases. Results from this study show that, among women with a pre-pregnancy weight < 91 kg, relative to women with no history of GDM, women with GDM had an elevated risk of developing CVD [aHR=1.4 (95% CI: 1.0-1.9)]. Also, given that Kaul et al. (141) report aHRs using the referent group of women with a pre-pregnancy weight < 91 kg and no GDM, for women with no GDM and weight ≥ 91 kg [aHR=1.5 (95% CI: 1.2-1.8)] and for women with GDM and weight ≥ 91 kg [aHR=2.1 (95% CI: 1.1-3.5)], we can estimate that among women

with a pre-pregnancy weight $\geq$ 91 kg, the HR for the association between GDM and CVD is similar to the association found in the present study (Kaul: 2.1/1.5 = 1.4). These results also suggest that overweight status does not modify the association between GDM and CVD.

The most recent study, conducted by McKenzie-Sampson and colleagues (146), followed women for up to 25 years after pregnancy. This follow-up time was similar to the follow-up time in the present study (up to 26 years) but was the longest follow-up time out of the eleven studies that examined the association between GDM and CVD. Follow-up in previous studies was limited to ten to twelve years after pregnancy. This study also had the largest sample size out of all the studies, including the present study. They observed 1,070,667 women in Québec, Canada and found that GDM was associated with a higher risk of CVD hospitalization, after adjustment for baseline age, parity, time period, and socioeconomic deprivation [aHR=1.75 (95% CI: 1.69-1.81)] (146). This estimate aligns with the estimate found in the present study, albeit it is slightly higher. This study, however, did not adjust for body mass. As previously mentioned, body mass has been shown to attenuate the association between GDM and CVD, which may partially explain why their estimate was slightly higher than the estimate found in the present study. This study, with its follow-up period spanning over two decades, may have been more likely to capture CVD than past studies with a shorter follow-up.

Several biological mechanisms may contribute to the elevated risk of developing CVD associated with GDM. It is plausible that GDM disrupts cardiometabolic function and can have permanent cardiovascular effects in the mother. More detailed explanations of these mechanisms can be found in Chapter 2.3.

Overall, these previous analyses, in addition to the present study, provide evidence for a positive association between GDM and CVD. The present study was able to address some of the

shortcomings of the previous studies and may have provided a more accurate estimate of the total effect of GDM on CVD.

6.3 Comparison with Previous Studies: Association between Gestational Diabetes Mellitus and Type of Cardiovascular Disease

Five studies examined the association between GDM and the risk of stroke and IHD as separate end points and reported results mainly consistent with the results of this study (7,140,143,145,146). All five studies found that GDM was positively associated with IHD (7,140,143,145,146), but only one study found that GDM was associated with stroke (146). The four studies that reported no association between GDM and stroke, in addition to the present study, may have been underpowered with respect to this relatively rare outcome. The number women without GDM who had a stroke and the number of women with GDM who had a stroke in the four studies that found no association between GDM and stroke are as follows: 31 and 19 (7); 520 and 33 (143); 2560 and 139 (140); 50 and 14 women (145), respectively. In the present study, 241 women with no GDM had a stroke later in life and 12 women with GDM had a stroke. In the study that found an association between GDM and stroke, 3498 women with no history of GDM had a stroke, while 283 women with a history of GDM had a stroke (146). It is possible that larger samples are needed to analyze the association between GDM and stroke with greater precision and to make accurate conclusions.

6.4 Comparison with Previous Studies: Mediation of the Association between Gestational Diabetes Mellitus and Cardiovascular Disease Risk by Type 2 Diabetes Mellitus

Results from three types of analyses can be used to assess mediation of the association between GDM and CVD by T2DM: assessment of the joint association between GDM and T2DM in relation to CVD; exploration of the effect of adjusting for T2DM on the effect estimate

for the association between GDM and CVD; and formal mediation analysis (162,163,166), which uses causal inference and allows an estimate of the proportion of the total association between GDM and CVD that is mediated by T2DM. The latter of the three types of analyses addresses shortcomings of the first two types of analyses that should lead to more accurate estimates and greater internal validity.

Two studies examined the joint association of GDM and T2DM with CVD as a means of assessing the extent of mediation by T2DM by dividing their cohort into four groups: no GDM and no T2DM; no GDM and subsequent T2DM; GDM and no subsequent T2DM; and GDM and subsequent T2DM (12,143). Retnakaran and colleagues (12) found that all groups exhibited an increased risk of CVD when compared to the reference group of no GDM and no T2DM, after adjustment for age, income, and region of residence. These findings suggest that women with GDM in whom T2DM does not develop are still at increased risk of developing CVD later in life [aHR=1.53 (95% CI: 1.26-1.86)] (12). These findings are not consistent with what was found by Tobias and colleagues (143), who found that women with a history of GDM but who did not develop T2DM did not experience an elevated CVD risk after adjusting for confounders, including pre-pregnancy BMI [aHR=1.30 (95% CI: 0.99-1.71)]. Women who did not have GDM but developed T2DM and women who had GDM and developed T2DM had greater than a four-fold increase in CVD risk [aHR=4.48 (95% CI: 2.25-8.91), aHR=4.04 (95% CI: 1.96-8.36), respectively].

The findings of the present study agree with the findings of the study conducted by Tobias and colleagues (143). In the present study, women who did not have GDM but developed T2DM and women who had GDM and developed T2DM had greater than a three-fold increase in risk of developing CVD [aHR=3.19 (95% CI: 2.52-4.04), aHR=3.64 (95% CI: 2.62-5.06),

respectively]. The findings of this study and the study conducted by Tobias and colleagues (143) suggest that the relationship between GDM and CVD can be explained by its progression to T2DM, after adjustment for confounders including body mass. It is possible that the lack of adjustment for body mass in the study conducted by Retnakaran and colleagues (12) could explain why women with GDM who did not develop T2DM still had an elevated risk of CVD. Body mass was adjusted for in the present study, as it was in the study conducted by Tobias and colleagues (143). Body mass was found to be a confounder of the association between GDM and CVD in the present study and others (7,140), so it is plausible that adjusting for it in the study by Retanakran et al. (12) would attenuate the association between GDM and CVD among women who had GDM but did not develop T2DM.

Five studies used the traditional approach to mediation analysis to assess whether T2DM mediated the association between GDM and CVD and found conflicting results (7,11,136,139,140). The traditional approach involved estimating the direct effect of GDM on CVD by adjusting for T2DM in one statistical model and not adjusting for T2DM in the other statistical model. Three of the five studies found that T2DM fully attenuated the association between GDM and CVD (11,136,139), while the other two studies found that a statistically significant association between GDM and CVD remained after adjustment for T2DM (7,140). By evaluating mediation by T2DM using the traditional approach, all five of these studies may be susceptible to three main sources of bias: T2DM-CVD confounding, GDM-T2DM interaction, and T2DM-CVD confounding affected by GDM, which may lead to inaccurate results. These five studies were also unable to report an estimate of the proportion mediated by T2DM as using the traditional approach does not allow for an accurate estimate (147).

The present study is, to my knowledge, the first study to conduct a formal mediation analysis of the association between GDM and CVD. The approach to mediation analysis used in this study should have, theoretically, reduced some of the potential bias previously mentioned and led to more accurate results. The result of this formal mediation analysis suggest that the effect of GDM on CVD appears to be almost fully mediated by T2DM, but this result should be corroborated in future studies using the newly developed formal mediation analysis methods.

6.5 Study Strengths and Limitations

A key strength of this study is that it had a large sample size (n=87,632) and that it was population-based, covering the entire province of Nova Scotia. Having a large sample size also resulted in precise effect estimates with narrow CIs. Since the data cover the entire province of Nova Scotia, the results could be generalizable to other areas of Canada that share similar characteristics. Further, the risk of reporting bias is reduced as these data were derived from administrative databases and were not self-reported. Self-report, where the individual recalls their diagnosis of GDM may result in inaccuracies and accuracy of recall of GDM may differ between women who developed and did not develop CVD, leading to bias. The exposure information found in the NSAPD for this study was based on diagnoses from qualified health care providers and is more likely to be accurate.

This study also addressed many limitations of other studies that examined the association between GDM and CVD. As previously discussed, these limitations mainly pertained to not adjusting for the appropriate confounders in the analysis, sometimes inappropriately adjusting for mediators, not conducting a formal mediation analysis, and using self-report data to ascertain CVD. Also, Retnakaran and Shah (12) may have introduced misclassification bias when they

chose to select a random pregnancy for those women with multiple pregnancies. By doing so, they may have missed some cases of GDM, leading to a wash-out and smaller power. I was able to account for all pregnancies in the analysis. Also, six studies (136,139-142,145) adjusted for hypertension, pre-eclampsia, and/or dyslipidemia; however, these conditions are on the causal pathway between GDM and CVD and are, therefore, likely mediators. As was discussed, adjusting for mediators may have biased the HRs that resulted from their analysis such that they did not properly estimate the total effect between GDM and CVD, leading the authors to draw flawed conclusions. This study addressed these issues by conducting a formal mediation analysis based on counterfactuals and marginal structural models that directly parameterize the natural direct and indirect effects of interest. Finally, two of the eleven previous studies used a cross-sectional design (7,144). The estimates obtained in these cross-sectional studies are susceptible to recall and survivor bias as GDM and CVD were ascertained by self-report and only included women who remain alive to participate in the study. As previously mentioned, this study addressed this limitation by using a retrospective cohort design with administrative data instead of self-report.

It is also important to identify the limitations of this study. A potential issue with this study is missing data on confounders such as weight and smoking status from the NSAPD. A proportion of pregnancies had this information missing. In the present study, multiple imputation using chained equations was used to replace missing values of covariates in 50 imputed datasets. The associations between GDM and CVD, GDM and stroke, and GDM and IHD were analyzed in these imputed datasets and were compared to results obtained in the complete case analysis. The similar aHRs obtained using both methods suggest that the missing covariate data do not introduce selection bias, although this statement is predicated by the assumption that the

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covariate data were missing at random. The results from the complete case analysis were shown due to their simplicity.

Other risk factors, not captured by the NSAPD or the administrative databases, may have potentially confounded the association between GDM and CVD. Ethnicity, race, history of PCOS, family history of T2DM and CVD, individual-level income, and other anthropometric measurements are potential confounders that are not recorded in these databases and as a result, were not adjusted for in the models.

Ascertainment bias may have been introduced as the databases contain information on diagnosed cases only and, therefore, cases that are not diagnosed by a physician may have been missed. A woman who was diagnosed with GDM may have had more frequent physician encounters after pregnancy than a woman who did not develop GDM due to potential health concerns related to the diagnosis and may, therefore, have been more likely to be diagnosed with T2DM or CVD than a woman without a history of GDM. I examined whether ascertainment bias was present by checking the proportional hazards assumption and found that this assumption was not violated.

False positives of the outcomes may have accumulated in this study as a woman who was recorded as having the outcome in one 2-year window would have been retained as having the outcome even if she did not have visits pertaining to the outcome in subsequent years. These factors could have potentially led to systematic error. Deriving data from administrative databases may have introduced misclassification bias. Billing practices may have differed between physicians, resulting in over-coding of certain diagnoses and under-coding of others. Also, diagnoses reported in administrative databases may not have been accompanied by documentation of the appropriate confirmatory tests and, therefore, validity of these databases to

define outcomes could not be assessed in this study. Cases of T2DM may have been missed if women had insufficient access to care, which may have resulted in non-differential misclassification of T2DM. Additionally, coding accuracy in administrative databases may have been negatively affected by clerical error, the inability of codes to describe conditions and procedures in detail, and omission of comorbidity codes due to perceived irrelevance to a visit (167).

As previously mentioned, the algorithm used in the present study to identify women who developed CVD was found to have a specificity of 98.0% in a previous study (155). The population in the present study had a low prevalence of CVD (1224 women) and, therefore, with this specificity, the positive predictive value may have been low. Also, this algorithm with a specificity of 98% for a diagnosis of CVD, which is a rare outcome, likely led to a dilution of the CVD positive group as some women who did not actually have CVD in the study period would have been classified as having CVD. Assuming that the degree of misclassification was not differential by GDM status, the likely result was an underestimation of the true association between GDM and CVD.

Some women diagnosed with GDM likely had undiagnosed T2DM before pregnancy and the postpartum screening recommended by Diabetes Canada was intended to pick up those cases. Women who were diagnosed within six months after delivery were excluded from the analysis. Although Diabetes Canada recommends women with GDM receive postpartum screening for T2DM up to six months after delivery, only a small percentage of these women do (168). Low adherence to these screening guidelines may have resulted in T2DM being diagnosed later in life when this condition was present prior to the pregnancy. Delayed diagnoses of T2DM may have potentially inflated the number of women who developed T2DM after GDM, resulting in an

over-estimation of the association between GDM and T2DM and an over-estimation of the proportion of the association between GDM and CVD that is mediated by T2DM.

The NSAPD does not contain information on homebirths that occurred with the assistance of a midwife prior to 2009, which may have resulted in missing data for these mothers. The characteristics of these women may have differed systematically from the women who gave birth in hospitals in Nova Scotia, resulting in selection bias. However, home births represented < 0.1% of all births in Nova Scotia (Irene Gagnon, Reproductive Care Program of Nova Scotia, Personal Communication) and, therefore, the omission of home births prior to 2009 unlikely affected the results of this study.

Overall, the limitations outlined were addressed in this study, if possible. Some of the limitations mentioned were trade-offs to using administrative databases that could not be reasonably addressed in this study.

6.6 Future Research Directions

Several studies have highlighted a positive relationship between GDM and the development of CVD later in life; however, this relationship is still poorly understood. It is still unclear whether this association is independent of body mass. Numerous previous studies evaluated the association between GDM and CVD in population-based cohorts, but follow-up was limited in most of these studies to twelve years or less. This is problematic as this is insufficient time for many of these CVDs to emerge and, therefore, these events may be missed in these studies. This would, as a result, limit the ability to assess if the association persists over time. Future studies should have long follow-up time to allow this investigation.

Results from the present study suggest that T2DM almost fully mediates the association between GDM and CVD; however, this is the first study to conduct a formal mediation analysis and its results should be confirmed in other observational studies. Future studies of the association between GDM and CVD should take advantage of recent advances in mediation analysis methods to obtain an estimate of the proportion of the association between GDM and CVD that is mediated by T2DM.

6.7 Study Significance and Impact

The results from this study could potentially influence decision making regarding public health policy and programs and could be used to guide clinical practice guidelines for care of women both during the pregnancy and after they give birth. The results of this study suggest that women with GDM have an elevated risk of developing CVD later in life compared to women without GDM. The increased risk of CVD associated with GDM provides justification for health care providers to recommend positive lifestyle changes from pregnancy onwards. Pregnancy is an opportune time for health care providers to develop a partnership with their patients who have GDM as women are often more conscious of their health behaviours during pregnancy and after birth and are amenable to positive lifestyle changes. Early patient engagement and health education may be effective at helping to reduce modifiable CVD risk factors and improve overall cardiovascular health.

The results of the mediation analysis, however, show that the association between GDM and CVD is almost entirely mediated by T2DM. Together, these findings support the incorporation of lifestyle and/or pharmacological interventions targeting the prevention of T2DM among women with a history of GDM. Health care providers should also be encouraged to

ensure that their patients who have a history of GDM are screened for T2DM postpartum. Health care providers should also inquire about whether a patient has a history of GDM and/or T2DM when assessing risk for CVD. A history of GDM and T2DM may warrant early screening and counseling to prevent CVD.

6.8 Conclusions

This thesis aimed to quantify the risk of subsequent CVD associated with GDM and to estimate the proportion of this association that is mediated by T2DM. Overall, the results suggest that women with a history of GDM have an increased risk of CVD later in life, but that this increased risk is nearly fully mediated by the development of T2DM. Collectively, these findings support increased screening and lifestyle counseling in women with prior GDM to improve cardiovascular health.

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