Excessive Gestational Weight Gain and Long-Term Maternal Cardiovascular Health

by

Franya Elizabeth Hutchins

BMus, Appalachian State University, 2005

MA History, Appalachian State University, 2008

MS Agricultural and Life Sciences, Virginia Polytechnic Institute and State University, 2014

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This dissertation was presented

by

Franya Elizabeth Hutchins

It was defended on

July 7, 2020

and approved by

Samar R. El Khoudary, PhD
Associate Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Janet M Catov, PhD
Associate Professor, Departments of Epidemiology
Graduate School of Public Health, University of Pittsburgh
Department of Obstetrics, Gynecology & Reproductive Sciences
UPMC Magee-Women's Hospital

Robert T. Krafty, PhD
Associate Professor, Department of Biostatistics
Graduate School of Public Health, University of Pittsburgh

Alicia B Colvin, PhD
Research Assistant Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Emma Barinas-Mitchell, PhD
Assistant Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh
Assistant Professor, Clinical Translational Science Institute
Director, Ultrasound Research Laboratory

Dissertation Advisor: Maria Mori Brooks, PhD
Professor, Departments of Epidemiology and Biostatistics
Graduate School of Public Health, University of Pittsburgh

Maria M. Brooks, PhD

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Franya Elizabeth Hutchins, PhD

University of Pittsburgh, 2020

Abstract

Cardiovascular disease (CVD) continues to be the leading cause of death in the United States despite decades of improvement in many risk factors. Public health literature often identifies the high prevalence of obesity as a contributor to the CVD burden. Excessive gestational weight gain (GWG) is emerging as a potential modifiable risk factor for obesity among those who give birth. However, there is no consensus as to whether excessive GWG contributes to CVD. Because obesity is a heterogeneous condition, it is important to evaluate the specific health sequela of a given risk factor. The overall objective of this dissertation is to investigate the role of excessive GWG in long-term maternal cardiovascular health. Using observational data, we estimated associations between excessive GWG and cardiovascular risk factors, and quantified the statistical bias around estimates. In the first aim of this dissertation, we estimated the association between number of births with excessive GWG and midlife BMI in a sample of parous participants in the multi-ethnic cohort Study of Women's Health Across the Nation. We found that each additional excessive GWG pregnancy was associated with increased maternal BMI at midlife independent of demographic, behavioral, and other reproductive factors. In aim 2, we quantified the potential statistical bias around these estimates to evaluate their susceptibility to common sources of systematic error. Using multiple imputation and misclassification-weighted regressions, we found that our estimates were generally robust to bias. In aim 3, we evaluate whether excessive GWG impacts atherosclerotic CVD risk score or chronic inflammation using 20 years of prospective

follow-up across midlife. We found that a history of excessive GWG was associated with a small but statistically significant increase in maternal CVD risk score, and moderate increase in inflammation.

Public health significance: Our findings underscore the importance of prenatal care in supporting long-term maternal health, and highlight inflammation as a potential pathway linking reproductive history to CVD. Further, we illustrate that observational data can provide valuable epidemiologic insights even in the presence of likely systematic error.

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1.0 Introduction

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in the United States. This is despite decades of improvement in some CVD risk factors such as smoking and cholesterol management. The continuing burden of CVD in the US is widely attributed to the increasing prevalence of obesity.

Over one-third of US adults have an obese body mass index, with the highest prevalence rate among women in their midlife. It is well established that those with obesity are at an elevated risk of CVD. Prevention of obesity, however, is challenging. Many of the known risk factors for adult obesity are difficult to intervene on. These include characteristics such as genetic susceptibility, childhood obesity, history of childhood abuse, and health disparities associated with race, ethnicity and socioeconomic status.

Excessive weight gain during pregnancy is emerging as a potential modifiable risk factor for midlife obesity among people who give birth. Gestational weight gain (GWG) is a vital component of healthy fetal development, but many pregnant people gain in excess of clinical recommendations. Epidemiologic studies have consistently shown excessive GWG in a single pregnancy to be positively associated with higher maternal weight years after the birth. However, there is no consensus on whether reproductive factors that are associated with obesity, such as GWG, are therefore associated with CVD risk. GWG has rarely been evaluated for association with long-term maternal cardiovascular health, with conflicting results.

The lack of a consistent connection between excessive GWG and cardiovascular risk may be due to several factors. Obesity is increasingly being understood as a heterogeneous condition. The cardiovascular risk of obesity may vary by the distribution of adipose tissue in the body or

other factors. It is possible that excessive GWG contributes to development of a lower risk phenotype of obesity with weakened effects on CVD. In addition, studies of reproductive history with long-term follow-up are vulnerable to multiple sources of statistical bias. Associations observed between excessive GWG and obesity may be influenced by systematic error.

The goal of this dissertation is to evaluate whether excessive GWG increases women's susceptibility to a high-risk phenotype of obesity. By applying a rigorous methodology, we aim to identify key associations on the potential pathway between excessive GWG, maternal midlife obesity, and CVD. We will also quantify statistical bias in the association between excessive GWG and midlife obesity.

This introductory chapter is organized as follows: First, section 1.1 discusses the epidemiology of each step in the potential pathway from excessive GWG to cardiovascular risk factors including obesity, and from risk factors to CVD. Section 1.2 provides a brief overview of the biological mechanisms driving the development of CVD. Special emphasis is placed on gaps in the literature regarding obesity's role in this process. Next, section 1.3 describes common obstacles and methodological solutions in researching health characteristics that develop over the life course. Finally, the dissertation's specific aims and conceptual model are presented in section 1.4.

1.1 Epidemiology of Excessive Gestational Weight Gain and Cardiovascular Disease

1.1.1 Prevalence of CVD and its primary risk factors in the US

Over 250,000 women die of CVD each year in the United States.[1] In fact, CVD remains the leading cause of death for US adults.[1] This is despite historic improvements in CVD risk factors such as total cholesterol and smoking in the population since the 1960's.[2,3] The definition of CVD generally includes atherosclerotic disease such as myocardial infarction, angina, revascularization, stroke, and peripheral arterial disease, as well as heart failure and atrial fibrillation.[4]

The primary risk factors for CVD are age, sex, race, hypertension, smoking, diabetes, and dyslipidemia.[5] Risk-enhancing factors include family history, elevated glucose, chronic inflammation, early age at menopause, and obesity.[4] Prior reductions in prevalence of hypertension and dyslipidemia have stalled in recent years. In 2015-2016, 29 percent of US adults had hypertension, with a majority of cases (52%) being uncontrolled.[6] In the same time period, 12.4 percent of adults had high total cholesterol ($\geq$ 240 mg/dL).[7] Meanwhile, diabetes and obesity prevalence have increased.[8-10] The current prevalence of diabetes among US adults is estimated at 14 percent, with 4.3 percent undiagnosed.[11] Finally, although smoking has dropped over the decades, 19.3 percent of US adults continue to be current smokers today.[12]

1.1.2 Obesity as a risk-enhancing factor for CVD

Obesity is a highly prevalent characteristic in the United States. Nearly 40 percent of US adults have an obese BMI, defined clinically as ≥ 30 kg/m^2.[13] Women in their midlife (40-59 years of age) have the highest rate of obesity of any age and sex group at 44.7 percent.[13] For some race

and ethnicity groups, these rates are even higher. Among US adults age 20 years and older, Black women have the highest prevalence of obesity at 54.8 percent, followed by Hispanic women at 50.6 percent, compared to the average of 41.1 percent for women overall.[13]

Having an obese BMI is associated with increased risk of coronary heart disease, ischemic stroke, and fatal cardiovascular disease.[14] Furthermore, obesity is linked to other CVD risk factors such as hypertension[15] and diabetes.[14] These risks and high prevalence in the population have made obesity a major public health concern.

However, the direct effect of obesity on CVD is not well understood. Obesity is highly comorbid with other risk factors such as diabetes and dyslipidemia. Previous literature including large pooled cohort designs have demonstrated that much of the effect of obesity on CHD events and stroke can be accounted for through other risk factors. A collaborative meta-analysis of 58 prospective studies representing over 200,000 participants found that individually, increasing BMI and waist circumference were both associated with hazard of incident coronary heart disease event or stroke. However, neither measure improved prediction beyond that of other risk factors such as diabetes, lipids, blood pressure, CRP, smoking, and sex.[16] A second collaborative meta-analysis of 97 prospective cohorts representing 1.8 million participants found that roughly half the coronary heart disease risk and 75 percent of the stroke risk attributable to BMI were mediated through blood pressure, cholesterol, and glucose.[17]

Although it is an established CVD risk factor in populations, obesity is not a consistent predictor on an individual level.[18] This may be due to interactions between obesity and metabolic health status. Individuals can be metabolically healthy or unhealthy at any BMI category.[18,19] Individuals with an obese BMI and a healthy metabolic profile are considered metabolically healthy obese (MHO) in some literature. The definition of metabolic health among those with

obesity varies, but generally refers to absence of hypertension, high fasting blood glucose, and dyslipidemia. Some definitions also include waist circumference, CRP, HOMA-IR, or cardiorespiratory fitness.[18,20,21] The proportion of those with obesity who are metabolically healthy varies greatly across studies because of heterogeneity in study populations and the lack of a consistent definition.[22,23]

Individuals with MHO may be at intermediate risk between those with a metabolically healthy normal weight phenotype and a metabolically unhealthy obese phenotype.[24] For example, a 2018 analysis of the Nurses' Health Study found that women of normal weight with a metabolically unhealthy phenotype were at greater risk of cardiovascular disease than women with an obese BMI who were metabolically healthy. Having a metabolically healthy phenotype was protective against cardiovascular disease in all BMI categories.[21] These results are consistent with a large meta-analysis of prospective studies.[25]

The controversial "obesity paradox" is a related but distinct phenomenon discussed in the literature. This refers to results found in multiple studies that associate an obese BMI with better prognosis among those with established cardiovascular disease.[26,27] Studies taking a causal approach however have suggested that this effect may be explained by residual confounding.[28-30]

1.1.3 Excessive gestational weight gain and maternal midlife obesity

Gestational weight gain (GWG) is a vital component of pregnancy. GWG reflects the development of the fetus as well as critical resources such as the placenta, amniotic fluid, and mammary glands.[31] The Institute of Medicine (IOM) has published clinical guidelines for the amount of weight gain needed to support a healthy pregnancy.[31,32] Total pregnancy weight gain is

characterized as “inadequate,” “adequate,” or “excessive” depending on pre-pregnancy BMI category.

Most pregnant people in the US gain outside of the recommended range for adequate GWG. Surveillance data report that 20.9 percent have inadequate gain and 47.2 percent have excessive gain.[33] Among people with an overweight or obese BMI prior to pregnancy, over 60 percent have excessive gain.[33] Cohort studies of pregnancy have also consistently observed over 40 percent of pregnant people to exceed the IOM guidelines.[34-38]

Previous studies have demonstrated an association between excessive GWG in a single pregnancy and long-term maternal weight. Multiple studies have estimated the impact of increased GWG on maternal weight at an average of 7 to 9 years following the birth. McClure et al[39] observed a 3.9 kg weight gain difference and odds ratio of 2.9 for obesity among women with excessive GWG compared to those with adequate GWG, adjusting for pre-pregnancy BMI. Davis et al[40] estimated a hazard ratio for obesity of 2.41 at an average 8 years of follow-up among over 3,000 women participating in the National Longitudinal Survey of Youth 1979. Additional evidence of the association between excessive GWG and increased maternal weight has been observed in various US populations[41-43] and a large Danish cohort.[44]

Some evidence supports an association more than fifteen years post-partum.[45-47] Using 18-years of follow up from the National Longitudinal Survey of Youth 1979, Cohen et al[46] found that maternal obesity prevalence was higher at midlife among women with a history of excessive GWG. Mamun et al[45] estimated a four-fold increase in the odds of obesity among women with excessive GWG compared to those without at 21 years following the index birth. Finally, a causal analysis by Abrams et al[47] estimated that prevention of excessive GWG in a woman’s first

pregnancy could reduce the prevalence of midlife obesity by 3 percent among White mothers (from 26.8 to 23.8%) and by over 6 percent among Black mothers (from 49.7 to 43.0%).

Despite these findings, significant gaps in knowledge linking excessive GWG to maternal obesity remain. This literature has consistently found an association independent of parity, socioeconomic status, and race. However, pregnancy complications, gestational age, and characteristics at the outcome measure such as physical activity or diet are rarely included. Few studies have considered whether weight characteristics across multiple pregnancies have a cumulative effect on maternal health.[46,47] None to our knowledge have considered GWG across all pregnancies in participants' reproductive history. If the impact is cumulative, prevention strategies could improve long-term maternal health even when implemented in later pregnancies. Furthermore, there is little research on whether the association between excessive GWG and later maternal weight varies across racial or ethnic groups. The association has rarely been stratified by race or ethnicity,[47] and no study to our knowledge based in the US has stratified by race or ethnicity in a cohort with representation of participants from any Asian background.

1.1.4 Excessive GWG and cardiovascular risk factors

Little research is available on GWG adequacy and maternal CVD risk factors outside of obesity. One cohort of 500 women with 8 years postpartum follow-up showed no significant difference in maternal triglycerides, LDL, total cholesterol, or metabolic syndrome prevalence between those with excessive versus adequate GWG.[39] A prospective study of 3,000 women found that those with excessive GWG were 1.47 times more likely to experience diabetes at 21 years post-partum compared to women with adequate GWG. The association was completely mediated through BMI at follow-up.[48] Conversely, analysis of a cohort of 800 women did not find an

association between continuous GWG amount and insulin resistance at a much shorter follow-up length of 3 years postpartum.[42]

1.1.5 Other reproductive factors and CVD risk

Many reproductive factors outside of weight can influence maternal health. Characteristics such as parity, pregnancy complications, and breastfeeding may confound or modify the effect of excessive GWG on CVD risk. Some characteristics have a direct effect on CVD risk, while others share risk factors with CVD. Understanding the potential relationships between these factors is critical to identifying the independent contribution of excessive GWG to CVD risk.

Parity has been shown to be positively associated with maternal insulin resistance later in life in multiple populations of women.[49-52] However, one large study found no association between parity and the odds of diabetes.[53] The literature on parity as an independent predictor for maternal CVD is conflicting.[54-58] A recent meta-analysis suggests that the relationship between number of births and cardiovascular mortality may follow a J-shaped pattern .[59] However, the parity literature is difficult to interpret because most studies do not account for GWG or pregnancy complications.

Pregnancy complications likely account for part of the CVD risk previously attributed to parity.[60] Increases in CVD risk factors such as triglycerides and inflammatory markers are expected in pregnancy[61] but generally return to prepregnancy levels postpartum.[62] In pregnancies with complications, however, vascular and endothelial dysfunction can continue after birth.[62] Complications such as preeclampsia and gestational diabetes are associated with later maternal CVD risk.[4,54,63-65] These conditions may contribute to CVD risk or, alternatively, serve as a marker of preexisting subclinical dysfunction.[62]

Experiencing a preterm birth is associated with maternal CVD risk factors,[66] and CVD events years after pregnancy.[67,68] Notably, the study of preterm birth is difficult because it shares risk factors with CVD including smoking, hypertension, and diabetes [61] as well as dyslipidemia.[69] However, an analysis of over 70,000 parous women in the Nurses' Health Study found an increased hazard of CVD events among those who had experienced a preterm birth, independent of other CV risk factors.[68] Additionally, there is evidence that maternal weight and GWG impact risk of preterm birth.[70-72] Inadequate GWG is associated with risk of preterm birth, but the association is stronger among underweight women.[70,72] Excessive GWG is not consistently shown to contribute to risk of preterm birth, but this may vary by race. [73,74]

Finally, breastfeeding has been associated with lower risk of diabetes,[53,75,76] and there is limited evidence that it is protective against CVD.[55,77] Although it is widely believed anecdotally that breastfeeding also improves postpartum weight loss, the literature does not consistently confirm this.[78,79] This may be because breastfeeding as an exposure is difficult to define consistently across studies.[79]

1.2 Identification of Biologic Pathways to Link Excessive GWG with CVD

1.2.1 Primary processes of CVD development

CVD can be characterized mechanistically as pathological restriction of blood flow. This primarily occurs due to atherosclerotic blockage or hypertensive damage to blood vessels. Dyslipidemia, inflammation, and elevated glucose can contribute to these processes. These risk factors are often comorbid, making the independent contributions difficult to quantify.

Atherosclerosis is the hallmark of many forms of CVD. The atherosclerotic process is driven by excess cholesterol in the blood stream and inflammation. Lipids including cholesterol are important components of all human cells. Being water insoluble, lipids must be attached to proteins to be transported through the body. The resulting lipoproteins are classified by density and have different effects on atherosclerosis. Low-density lipoproteins (LDL) carry cholesterol from the liver to peripheral tissue. However, LDL and other pro-atherosclerotic lipoproteins that are not taken up by peripheral tissue can remain in the circulatory system and become toxic.[80] These can also enter blood vessel endothelium and become oxidized. An immune response is then triggered to clear the damaged lipoprotein.[81]

Inflammation is a key process in atherosclerotic development and progression. As immune cells try to clear modified lipoproteins in the endothelium, large foam cells build up. Local inflammation is induced including immune cell cytokine release resulting in smooth muscle cell migration from the media to the intima and subsequent proliferation. The accumulation of lipid-laden foam cells and smooth muscle cells within the intima eventually results in a growing lesion or atherosclerotic plaque. Chronic inflammation can also disrupt existing plaques, which can lead to thrombosis.[82] Oxidative stress and inflammation have a cyclic relationship that continues the process.[83,84]

Hypertension is also a major driver of CVD. The heart, blood vessels, and kidneys all have a role in the regulation of blood pressure. Heart rate and blood vessel dilation impact blood pressure change in the short term.[85] In contrast, long-term changes in blood pressure involve renal function or vascular remodeling including media thickening.[86] When blood pressure increases consistently outside of the normal range—systolic > 140 or diastolic > 90 for adults under age 60 [87]—this is considered hypertension. Shear stress on the vascular endothelium due to increased

blood pressure may initiate atherosclerotic lesion development.[82] However, hypertension also contributes to CVD outside of the atherosclerotic process as thickening and stiffening of the arterial walls contribute to impaired blood flow. Hypertension is also a cause of hemorrhagic stroke and a risk factor for atrial fibrillation.

Finally, diabetes and elevated glucose are also contributors to CVD. Individuals with diabetes are more likely to have dyslipidemia, hypertension, and advanced atherosclerosis compared to non-diabetic individuals. This may be due to increased inflammation, oxidation, or macrophage infiltration, triggered by hyperglycemia.[88,89] Hyperglycemia independent of diabetes status contributes to increased oxidation that can produce vascular damage. Hyperglycemia has also been observed to increase lipolysis, releasing fatty acids into the circulation.[89]

1.2.2 Measuring cardiovascular risk

An ongoing project in CVD prevention is the development of clinical measures to capture the multi-faceted process of atherosclerotic disease. Quantifying the contributions of dyslipidemia, hypertension, diabetes, and inflammation as individual risk factors is cumbersome and difficult to interpret. Cardiovascular risk scores are a practical approach to summarize CVD risk. Validated risk scores provide a single measure to concisely represent CVD risk in epidemiologic research, or guide patient decision-making in a clinical setting.

The atherosclerotic CVD (ASCVD) risk score is based on the most recent literature on the impact of traditional CVD risk factors.[5] Developed by the American Heart Association and the American College of Cardiology, the ASCVD risk score measures the 10-year risk of developing a first atherosclerotic CVD event. Events include nonfatal myocardial infarction, coronary heart disease death, and fatal or nonfatal stroke. The score calculation is derived from 5 community-

based cohorts of Black and White participants and is comprised of diabetes status, age, total cholesterol, high density lipoprotein cholesterol, systolic blood pressure, blood pressure medication use, and current smoking status. Each component is multiplied by a race-specific coefficient, summed for a total score, and transformed by an overall race-specific formula.[5] The ASCVD score has been externally validated as predictive of CVD events.[90] It has also demonstrated good calibration to discriminate risk at the decision threshold of 7.5% in both women and men,[90] and it is an appropriate measure of absolute and relative CVD risk.

1.2.3 Biology of obesity development

Obesity is characterized by excessive accumulation of adipose tissue. Total adipose tissue as a percentage of body weight varies across individuals, ranging from 5 to 60 percent.[91] Adipose tissue is comprised of various cell types, categorized as adipocytes, preadipocytes, and non-adipocyte cells. Adipocytes are the main cells of adipose tissue, and store energy in the form of triglyceride droplets.[92] Non-adipocyte cells in the tissue include macrophages, immune cells, fibroblasts, connective tissue, vasculature, and neural tissue. Excess energy intake causes adipose tissue volume to increase as free fatty acids and glycerol are converted to triglycerides and stored.

Individuals show heterogeneity in their adaptive response to a continued positive energy imbalance.[93] Adipose tissue gain is determined by an individual's total energy expenditure rate. Total energy expenditure is comprised of resting and active energy expenditure, and varies across individuals. Further, weight gain can increase the resting metabolic rate, but the degree of metabolic change depends on the relative difference in adipose tissue volume versus skeletal muscle.[93] Gene-environment interactions can also impact the amount of adipose tissue that develops in the presence of overnutrition.

External factors can contribute to individual differences in adiposity. Physical activity can moderate weight gain through epigenetic changes.[94,95] Changes in gene expression may also be triggered by diet. A high fat diet has been shown to modulate expression in tissues such as liver, adipose, muscle, and hypothalamus.[96] Some studies have suggested a high sugar diet may lead to changes in transcription in liver tissues.[96] Finally, exposure to some environmental chemicals including certain pesticides, plasticizers, and endocrine disruptors, may increase individual susceptibility to obesity.[96]

1.2.4 Contributions of obesity to CVD

Biologically, obesity may contribute to atherosclerotic development through inflammation. Adipocyte cells secrete adipokines—biologically active molecules including proteins and cytokines. Many adipokines have established metabolic or immune roles.[92] Adipokines include both pro- and anti-inflammatory proteins, as well as MCP-1, which attracts macrophages.[97] Important adipokines include leptin, adiponectin (considered anti-atherogenic and anti-inflammatory), IL-6, and TNF-alpha.

Pro-inflammatory cytokines such as leptin promote vascular atherosclerosis.[98-100] Similarly, IL-6 and TNF-alpha have been shown to increase vascular inflammation and decrease insulin signaling.[97] Immune cells also contribute to endothelial dysfunction and oxidative stress in the development of atherosclerosis.[100] Processes common to adipose tissue and atherosclerosis are infiltration of macrophages, activation of T cells, and production of cytokines.[101] Inflammation may activate other factors contributing to a positive feedback loop.[101]

Adipose tissue contributes to insulin resistance in liver and skeletal muscle tissue. Triglycerides stored in adipocytes can be decomposed into free fatty acids and glycerol through

lipolysis. These components are then released from the adipocyte. Free fatty acids are bound to albumin and routed to muscle, liver, or another adipocyte through reesterification.[91] Lipid load in the liver can drive hepatic insulin resistance.[102] Accumulation of lipids in muscle cells can exceed cellular ability of lipid oxidation. This can lead to the development of toxic lipid intermediates that impair insulin signaling. When this occurs, increased insulin resistance is then compensated by increased insulin secretion.[102] Glycerol released from lipolysis is sent to the liver and converted into glucose.[91]

Finally, obesity also contributes to hypertension through multiple pathways. Increases in insulin, leptin, and renin stimulate the sympathetic nervous system.[15] In addition, obesity triggers increased renal reabsorption of sodium through multiple mechanisms.[15] Both pathways can result in long-term increase in blood pressure.

1.2.5 Obesity phenotypes

The most prominent theory for the variation of health risk among those with obesity is subcutaneous versus visceral adiposity phenotypes. When exposed to a positive energy imbalance, individuals are heterogeneous in their physiological capacity to store excess energy, both in how much and where fat is deposited.[103] Subcutaneous adipose tissue (SAT) is the first destination for storage.[97] Major subcutaneous stores are abdominal, gluteal, and femoral (thigh). Abdominal SAT accumulates between the skin and peritoneal membrane in two layers, superficial and deep, that are divided by connective tissue.[91]

When SAT storage capacity is exceeded by amount of energy imbalance or impaired by genetics or environmental stressors, triglycerides are deposited in other areas, which can lead to accumulation of visceral adipose tissue (VAT).[104] VAT accumulates around the digestive organs

in the intraperitoneal and retroperitoneal areas within the abdominal cavity.[91] This includes the omentum, a store of adipose tissue attached to the stomach within the peritoneal cavity. VAT can also be stored in the retroperitoneal area, i.e. along the posterior abdominal wall, near the kidneys.[103]

Subcutaneous and visceral adipose tissue vary in vascularity, cell size, receptors, and chemical secretion. VAT is more vascular and more heavily innervated than SAT. VAT is also more metabolically and immunologically active. It is more likely to be infiltrated by immune cells, so more often secretes proinflammatory, proatherogenic cytokines as well as free fatty acids.[91,102] Conversely, SAT is more active in absorbing free fatty acids and triglycerides.[102]

VAT is more consistently associated with dyslipidemia, hypertension, atherosclerosis, adipocytokine imbalance, and inflammation than SAT.[103] Obesity that presents with more VAT is associated with impaired insulin sensitivity and increase production of VLDL.[22] Because of its location within the abdominal cavity, venous blood from VAT drains to the liver via the portal vein. SAT venous blood drains into systemic circulation. Therefore, adipokines and free fatty acids excreted by VAT have direct route to the liver.[26,97] Retroperitoneal fat may be particularly important for hypertension due to its proximity to the kidneys.[103]

Notably, SAT contained in the lower body (gluteal and leg) has been observed to be inversely associated with CVD risk factors and events.[26,103] Furthermore, while lifestyle-based weight loss improves metabolic function, removal of SAT (e.g. liposuction) has not shown benefits.[91]

Adiposity that favors VAT accumulation compared to SAT is also described as the abdominal obesity phenotype. Despite the evidence in support of the VAT hypothesis, measures that differentiate between visceral and subcutaneous adipose tissue have not shown consistent

advantage for predicting metabolic risk.[102] Further, while VAT is generally associated with atherosclerotic development, studies vary on whether association remains after adjusting for other risk factors.[105] The clinical utility of including VAT measures to identify high risk individuals has not been established conclusively.[105]

An alternative theory of heterogeneous obesity related to the abdominal obesity phenotype focuses on ectopic adipose tissue. Some researchers argue that VAT is not the driver of risk but a marker of accumulation of more bioactive ectopic adipose tissue deposition. Ectopic adipose tissue accumulates as small depots where triglycerides are not physiologically stored, including the liver, pancreas, heart, and skeletal muscle.[91,103] Lipotoxicity in these tissues causes insulin resistance.[91] Ectopic adipose tissue in the heart (epicardial AT) has bidirectional communication with the myocardium.[106] Most but not all studies of epicardial AT have found association with progression of atherosclerosis.[105] Similarly, interhepatic ectopic adipose tissue is closely linked to insulin resistance in liver, skeletal muscle, and adipose tissue.[102] Obesity with higher hepatic fat has been linked to type-2 diabetes and atherosclerosis.[22]

Adipose tissue accumulates both through the generation of new adipocytes (hyperplasia) and growth in cell size of existing adipocytes (hypertrophia). A third theory proposes that adipose tissue growth via hypertrophia induces higher CV risk compared to hyperplasia. Small adipocytes actively absorb free fatty acids but over time will grow larger. These enlarged adipocytes become insulin-resistant and promote lipolysis. Enlarged adipocytes show imbalanced adipocytokine production that favors pro-inflammatory factors. This explanation is also related to the VAT theory, as SAT has a greater ability to generate new preadipocytes compared to VAT [91]. VAT is also more likely to contain larger, older adipocytes.[97] Hypoxia in adipose tissue induced by the expansion of adipocytes may also contribute to inflammation.[106,107]

Finally, some researchers propose that the heterogeneity of cardiovascular risk imparted by obesity is due to metabolic compensation in other tissue. For example, some researchers propose that physical fitness is a protective factor for metabolic health regardless of weight. However, there is not consistent evidence supporting this.[18] A related theory is metabolic capacity versus metabolic load. This states that the metabolic load of total adipose tissue may be offset by the metabolic capacity of fat-free mass in an individual. Body composition and cardiometabolic risk may be inversely linked through skeletal muscle mass. This is a compelling idea but not supported in studies comparing fat and fat-free mass in similar weight individuals.[102]

1.2.6 The contributions of excessive GWG to obesity and obesity phenotypes

It is biologically plausible that excessive GWG could contribute to increased maternal weight after birth. Maternal components such as total body water, protein, and adipose tissue account for approximately 65 percent of weight gained during pregnancy.[31] GWG amount correlates highly with fat accrual, and over 40 percent of the maternal tissue gained is estimated to be adipose tissue.[31] According to the IOM's 2009 review, the range of mean GWG among women with normal prepregnancy BMI is 10 to 14 kilograms. Women with overweight and obese prepregnancy BMI are generally observed with lower mean GWG, between 8 to 12 kilograms in previous studies.[31] Recently, several large multiethnic US cohorts and record reviews have found similar ranges among women with singleton live births.[108-110]

Weight increase in non-pregnant individuals depends on an imbalance of energy intake with resting and active energy expenditure. During pregnancy, however, there is evidence that both resting energy expenditure and weight—including fat mass—increase without a corresponding increase in energy intake.[111,112] The mechanisms behind this phenomenon are not well understood.

Pregnant individuals with a greater increase in resting energy expenditure have been observed to have less fat mass accrual compared to those with a more moderate increase.[113]

Maternal fat accretion during pregnancy is mainly subcutaneous and deposited in the upper and lower trunk.[31] However, there is some evidence that excessive GWG favors adipose tissue, including VAT. In a cohort of 49 pregnant women with overweight and obese prepregnancy BMI, amount of weight gain correlated positively with fat mass change.[114] Those with excessive GWG had a greater increase in fat mass compared to those with adequate GWG (5.2 kg versus 0.2 kg). In a prospective study of 52 pregnant women with and without gestational diabetes, those with a lean BMI gained a similar quantity of fat mass and lean body mass to those with an obese BMI.[115] Based on skinfold measures, most of the increase in adipose tissue during pregnancy developed in the upper thigh, suprailiac (waist), and costal (rib) regions. Lean women accrued more of their adipose tissue in peripheral depots compared to obese women who gained more centrally, in the suprailiac region.[115] Bone mineral and body water also increase during pregnancy and should be accounted for in measures of body composition. However, total body water accumulation during pregnancy does not appear to differ by prepregnancy BMI, while fat mass accumulation does.[31]

In the postpartum period, weight retention is positively correlated with total GWG[116] and total fat mass gain during pregnancy, but not with fat free mass gain.[117,118] Postpartum weight retention favors fat accumulated in the trunk, and may include VAT.[31,119] In a cohort of 41 pregnant women with live, singleton births in Korea, total body water and fat free mass decreased from day 2 following delivery to 6 weeks postpartum. Notably, fat mass and VAT area increased despite overall decrease in weight.[120] Weight gain in the postpartum period was also observed in a small cohort of US Black women.[121]

In a group of 93 pregnant women participating in a substudy of the Toronto Abdominal Visceral Obesity Study, visceral adipose tissue depth increased from mean (sd) of 4.1 (1.7) cm at week 11-14 gestation to 4.5 (1.8) cm at 6-12 weeks postpartum. There was not strong agreement between weight retention and VAT in this study. While 23 percent of women increased BMI from first trimester to the postpartum follow-up but lost VAT depth, 12 percent decreased in BMI but showed but gained VAT depth. An increase in VAT depth was associated with higher HOMA-IR at follow-up than an increase in BMI.[122]

Finally, a cohort of 302 pregnant Black and Dominican women in New York looked at long-term maternal body fat. This study found a positive association between excessive GWG and maternal percent body fat at 7 years postpartum. Further, the study reported a significant, negative interaction between excessive GWG and prepregnancy BMI on maternal body fat at follow-up.[43]

1.3 Challenges in Studying the Long-Term Effects of Reproductive Health

1.3.1 Potential sources of systematic error

While nearly all modern epidemiologic studies present estimates of random error such as confidence intervals, the quantification of systematic error is rare in the literature.[123] This is despite the fact that sources of systematic error including uncontrolled confounding, selection bias, and measurement error are common in practice. Systematic error (bias) has the potential to misrepresent the strength of an association, or even lead to incorrect inference. Quantitative bias analysis allows epidemiologists to evaluate the direction, magnitude, and potential uncertainty around systematic error.[124]

Long-term followup in a study allows for the prospective observation of outcomes that develop over time. However, a significant drawback to longterm studies is participant loss. If participants do not return to later study visits due to factors related to the exposure and outcome of interest, estimates of association can be biased. This is often the case in epidemiologic studies. Demographic characteristics associated with health outcomes, as well as worsening health, are commonly related to participant attrition.[125-127] This is considered a form of selection bias.

A second potential source of bias in these data is recall bias. Retrospective self-report is a common method for collecting data on reproductive history.[128] Characteristics such as GWG are practical to gather retrospectively, especially if information on more than one pregnancy is needed. However, the utility of retrospective self-report relies on the accuracy of participant memory. Self-report of pregnancy weight can be valid and reliable[129,130] but longer recall time can reduce the quality of these measures.[128]

Women often underestimate pre-pregnancy weight and overestimate GWG.[128] Some women who had adequate GWG are therefore misclassified as having excessive GWG. This results in overestimation of the prevalence of excessive GWG in the epidemiologic literature. A meta-analysis by Headen et al. found moderate misclassification of GWG adequacy in studies relying on maternal recall of weight.[128] Among studies measuring pregnancy weight characteristics multiple years after birth, the mean deviation from the true value was less than 1 kg. However, the magnitude of error varied widely among women and was significantly greater among those with higher BMIs and those of minority race/ethnicity.

1.3.2 Multiple imputation to address bias due to missing data

Multiple imputation is an established method to address selection bias.[131] This approach generates datasets with created values for missing data. Imputation is typically based on a model for the underlying distribution of the missing values. Each generated dataset is used in a separate analysis model, and parameters from these models are averaged to produce a single set of pooled estimates. Multiple imputation can reduce bias in cases where data are missing from the outcome, exposure, or covariates.[132]

When data are missing across more than one variable, values can be imputed through chained equations.[133] Multiple imputations by chained equations (MICE) is a flexible approach in which missing values are imputed sequentially for each variable conditional on all other variables in the model. These sequential imputations are traditionally regression-based models. Regression-based MICE can accommodate imputation of continuous, binary, and categorical variables. However, it requires specification of the distribution of each variable to be imputed.

Multiple imputation assumes that values are missing at random. Missing at random describes a study in which the characteristics related to having missing data have been observed.[131] If an unobserved characteristic is associated with having missing data after conditioning on observed data (i.e. data are missing not at random), complete case analysis and use of multiple imputation may yield biased results. Therefore, the mechanisms driving missingness in a given study must be well understood and relevant characteristics observed.

An alternative method to address missing data is inverse probability weighting, in which the investigator specifies a model to predict the probability of having complete data. The resulting probabilities are then used to create a weighted pseudo-population with complete data that reflects the full study population. Associations estimated from the pseudo-population represent what

would have been observed if no one had been censored by loss to follow-up. MICE may be preferred in applications where only a few persons with a given set of characteristics have complete data, as predicted probabilities of missingness can result in extreme weights. Secondly, weighted analysis models use only observed data, and are therefore less efficient than estimation under multiple imputation.

Multiple imputation is reasonably robust in generating unbiased estimates when the imputation model is misspecified.[131] One exception, however, is the imputation of non-linear relationships between variables. Common approaches such as regression-based and predictive mean matching imputation do not always perform well in the presence of interactions.[134-137] Interactions and polynomial terms may not be appropriately represented in imputed data. This caveat is important in modeling the effect of GWG adequacy on maternal health. Preliminary analysis suggests that history of preterm birth is an effect modifier of GWG adequacy on midlife obesity.

Proposed solutions include the "just another variable" technique and non-parametric approaches such as decision trees. In the "just another variable" approach, the interaction term is included in imputation models as its own variable. This approach can produce unbiased estimates from imputed data when one component of the interaction is missing.[134,138] However, methods in the literature do not address interactions in which both variables are missing together. In these data, preterm birth status is missing in women with missing GWG adequacy.

Decision trees, also known as recursive partitioning, predict missing values by splitting observed data into similar subsets. Subsequent splits are conditional on previous splits. Because variables can be reused in multiple splits, interactions present in the observed data are represented. Further, trees do not require any distributional assumptions. Decision trees have been used in

imputation, although often with a single resulting imputed dataset.[136,139-142] These approaches alone may be more biased than parametric MICE.[143] Furthermore, the creation of a single dataset fails to account for the uncertainty of missing values.[139] However, incorporating tree-based imputation within MICE can improve performance. This approach has been shown to result in less biased estimates compared to regression-based MICE when interaction effects are hypothesized.[136,143] This approach has a number of strengths, as it can impute missing values in multiple variables, accommodate interactions, and account for uncertainty around imputed values.

1.3.3 Quantification of recall bias

Analytic options to evaluate self-report include validation studies, reliability estimates, and sensitivity analysis. Validation studies require an objective or gold-standard measure to compare with self-reported values. Alternatively, reliability estimates require multiple occurrences of the same measure per participant. Formulas for reliability include percent agreement and the Kappa statistic, which represents the proportion of observed agreement between repeated measures beyond that expected by chance.

Validation and reliability analysis describe the level of confidence an investigator may have in a measure. To quantify the impact of bias on estimates of association, however, a sensitivity analysis is necessary.[144] Sensitivity analysis for misclassification of the exposure involves choosing parameters of sensitivity and specificity for the measure. To include multiple covariates, sensitivity and specificity or positive and negative predictive values can be used to create weights for observed combinations of exposure and outcome.[145] Weights are applied to multivariate logistic regression to estimate bias-adjusted associations given each set of parameters specified with jackknife estimates of standard error.[145,146] In a fixed-parameter approach to

sensitivity analysis, single values are specified for each model. In a probabilistic approach, the investigator specifies distributions around chosen values to account further for uncertainty of the parameters.[144,147] Either approach can handle non-differential or differential misclassification.[123]

Sensitivity analyses are highly dependent on the bias parameters chosen by the investigator. Results can vary widely depending on sensitivity and specificity values or distributions.[144] Providing parameters close to those of the measurement of interest is required to approximate the "true" association.[123] Because the accuracy of a measure in a specific cohort is unknown without additional validation data, it is important to obtain documented rates from the literature when possible.

1.3.4 Data Source: The Study of Women's Health Across the Nation

This dissertation uses data from the Study of Women's Health Across the Nation (SWAN), which is an ongoing prospective, multiethnic, multi-center study of women in midlife and later life. The 22-year study has been an important source of research on the menopausal transition and reproductive health.[148-150] Notably, it is one of the few cohort studies that captured lifetime reproductive history in US women. SWAN's many strengths include an ethnically and geographically diverse population and longitudinal measurement of large array of characteristics. The main areas of study focus are menstrual patterns and reproductive hormones, menopausal symptoms, psychosocial characteristics, cardiovascular measures, physical functioning and activity, cognitive functioning, bone health, medications, and sleep.

The SWAN cohort is made up of 3302 women enrolled from seven cities: Boston, MA, Chicago, IL, Detroit, MI, Los Angeles, CA, Oakland, CA, Newark, NJ, and Pittsburgh, PA. All sites enrolled women who identified as Non-Hispanic White as well as women from one additional

race or ethnic group: Black, Hispanic, Japanese, or Chinese. Since enrollment began in 1996, SWAN has conducted a screening interview, baseline visit, and 16 follow-up visits. (Please see Appendix A for study acknowledgements).

Selection bias is a potential concern in SWAN data. Over the 16 follow-up visits, women who return to the study are systematically different from those who leave. Our preliminary analysis suggests that nearly one third of the women who reported having live births in the baseline interview are missing from the reproductive history questionnaire, which was conducted at a later study visit. The women with the missing exposure data are different from those with full data in a number of ways including race/ethnicity, BMI, and smoking status.

These data may also be impacted by recall bias. Because the SWAN reproductive history questionnaire was given roughly 25 years following the participants' last birth, our measure of GWG adequacy is vulnerable to systematic error. If women with obesity at midlife are more likely to be misclassified with excessive GWG than other parous women, bias could account for some or all of the association observed. In this case, both absolute and relative measures of association may be biased.

1.4 Specific Aims and Conceptual Model

1.4.1 Specific Aims

Specific Aim 1: To evaluate the number of excessive gestational weight gain (GWG) pregnancies as a predictor of obesity in early midlife. Excessive GWG will be defined as a pregnancy resulting in live birth with reported weight gain above IOM guidelines for singleton births.

Hypothesis 1: The number of excessive GWG pregnancies will be positively associated with odds of obesity in early midlife (age 42 to 52), independent of other reproductive factors, physical activity, diet, and socioeconomic status. Analysis will also test whether the association varies by race/ethnicity or parity.

Specific Aim 2. Estimate potential bias in estimates of association between GWG and midlife obesity.

Sub-aim 2a: Estimates of association between excessive GWG and obesity will be evaluated for selection bias due to participant attrition using multiple imputation of missing exposure values.

Sub-aim 2b: Estimates of association between excessive GWG and midlife obesity will be evaluated for misclassification of the exposure using fixed-parameter sensitivity analysis of bias.

Specific Aim 3. To evaluate the long-term metabolic and cardiovascular impact of GWG.

Hypothesis 2: A history of excessive GWG will be positively associated with atherosclerotic disease risk score at a mean follow-up of 17 years following early midlife, through a pathway largely mediated by midlife obesity.

Hypothesis 3: A history of excessive GWG will be positively associated with inflammation measured by C-reactive protein at a mean follow-up of 17 years following early midlife, through a pathway largely mediated by midlife obesity.

2.0 The Effect of Gestational Weight Gain across Reproductive History on Maternal Body Mass Index in Midlife: The Study of Women's Health Across the Nation

Franya Hutchins[1], Barbara Abrams[2], Maria Brooks[1], Alicia Colvin[1], Tiffany Moore Simas[3], Milagros Rosal[4], Barbara Sternfeld[5], Sybil Crawford[6]

1. Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2. Division of Epidemiology, Graduate School, School of Public Health, University of California, Berkeley, Berkeley, CA; 3. Department of Obstetrics and Gynecology, University of Massachusetts Medical School, Worcester MA; 4. Department of Quantitative Health Sciences, University of Massachusetts Medical School, Worcester MA; 5. Division of Research, Kaiser Permanente, Oakland, CA; 6. Department of Medicine, University of Massachusetts Medical School, Worcester MA.

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2.1 Abstract

Background: Excessive weight gain during pregnancy is common and has been shown to be associated with increased long-term maternal weight. However, less is known on whether there is a cumulative effect of excessive gestational weight gain (GWG) over multiple pregnancies.

Methods: Data from the Study of Women's Health Across the Nation were used, restricted to parous women with no history of stillbirth or premature birth. The effect of the number of excessive GWG pregnancies on BMI in midlife (age 42 to 53) was analyzed using multivariable linear regression. Fully adjusted models included parity, inadequate GWG, demographic, and behavioral characteristics.

Results: The 1181 women included in this analysis reported a total of 2693 births. Overall, 466 (39.5%) were categorized as having at least one pregnancy with excessive GWG. The median BMI at midlife was 26.0 kg/m2 (IQR: 22.5, 31.1). In fully adjusted models, each additional pregnancy with excessive GWG was associated with 0.021 higher estimated log BMI (p=0.031). Among women with 1 to 3 births, adjusted mean (95% CI) BMI for those with 0, 1, 2, and 3 excessive GWG pregnancies was: 25.4 (24.9, 25.9), 26.8 (26.1, 27.5), 27.5 (26.6, 28.4), and 28.8 (27.3, 30.5), respectively.

Conclusion: In this multi-ethnic study of women with a history of term live births, the number of pregnancies with excessive GWG was associated with increased maternal BMI in midlife. Our findings suggest that prevention of excessive GWG at any point in a woman's reproductive history can have an impact on long-term maternal health.

2.2 Introduction

More than one in three adults in the United States have obesity, with the highest prevalence among women in midlife.[151] Those with obesity are at an elevated risk of adverse health outcomes including type 2 diabetes and fatal coronary heart disease.[152] Prevention of obesity is complicated

by social, behavioral, and biological factors.[153] Therefore, the identification of modifiable risk factors is a key public health concern.

A potential opportunity for obesity prevention prior to midlife is during pregnancy. Excessive gestational weight gain (GWG) is common[33] and is associated with long-term weight.[154] Findings from cohort studies suggest that over 40 percent of pregnant women exceed clinical guidelines for GWG adequacy.[36,37] This can have a long-term impact on maternal health. Excessive GWG has been linked to increased weight[43] and higher odds of obesity[39,40] over five years after pregnancy. Some evidence supports an association remaining more than fifteen years.[45,46]

While the current literature consistently shows a positive association of excessive GWG in a single pregnancy with maternal weight later in life, the impact of GWG over a woman's entire reproductive history is not well understood. Few studies have considered whether weight characteristics across multiple pregnancies have a cumulative effect on maternal health.[46,47] None to our knowledge have estimated the effect of GWG adequacy in all of a woman's pregnancies while controlling for lifestyle factors. The Study of Women's Health Across the Nation (SWAN) provides an opportunity to incorporate the full reproductive history of participants. The 22-year prospective SWAN study has been an important source of data on the menopausal transition and reproductive health.[148-150] It is one of the few cohort studies that captured lifetime reproductive history in US women. The goal of this analysis was to evaluate how GWG adequacy across all a woman's pregnancies contributed to obesity in midlife among primiparous and multiparous SWAN participants.

2.3 Methods

2.3.1 Participants

SWAN Cohort: This analysis involves a subset of women participating in the Study of Women's Health Across the Nation (SWAN), which is an ongoing prospective, multiethnic, multi-center study of women over the menopausal transition. The SWAN cohort is made up of 3302 women enrolled from seven cities: Boston, MA, Chicago, IL, Detroit, MI, Los Angeles, CA, Oakland, CA, Newark, NJ, and Pittsburgh, PA. Enrollment began in 1996 with the following primary eligibility criteria: age 42 to 52, having at least one menstrual period in previous 3 months, no exogenous hormone use in previous three months, intact uterus, at least one ovary, and self-identification with a designated racial/ethnic group recruited by site. All sites enrolled women who identified as Non-Hispanic White as well as women from one additional race or ethnic group: Non-Hispanic Black, Hispanic, Japanese, or Chinese. More information on the sampling strategy for SWAN has been published previously [155]. IRB approval was obtained with each site institution and written consent given by all participants.

Data Collection: A comprehensive reproductive history questionnaire was administered at the thirteenth follow-up visit (conducted in 2011 to 2013), which included prepregnancy weight, GWG amount, and gestational age for each live birth. Although SWAN also collected GWG amount for each pregnancy at the baseline visit (conducted in 1996 to 1997), prepregnancy weight and gestational age was not assessed at that time and all reproductive characteristics in our primary analysis were obtained from the visit 13 questionnaire.

Analytic Sample: The goal of this analysis was to evaluate the association between excessive GWG and midlife obesity among women with a history of term, singleton, live births.

Therefore, the analytic sample was drawn from women who reported 1 or more live births at term in their lifetime (n=2732). Women with a history of anorexia, bulimia, or thyroid disorders (n=303) were excluded.

Of the 2429 women reporting live birth(s) with no history of disordered eating or thyroid disease, 1639 completed a reproductive history questionnaire at SWAN follow-up visit 13. Those with multigestational births or pregnancies that lasted 5 months or longer resulting in stillbirth or premature birth were excluded (n=263). Additionally, women with missing outcome data (n=14), missing prepregnancy or gestational weight data for more than one pregnancy or for their only pregnancy if primiparous (n=73), as well as one participant who reported a birth after the outcome of midlife BMI was measured were excluded. Two additional women who reported GWG outside likely biological plausibility as defined in previous literature were excluded [156,157]. In sensitivity analyses, participants with missing covariate values (n=105) were included using mean imputation. Estimates for main predictors did not differ meaningfully between the complete case and imputed models (results not shown). Results presented are from complete-case analyses. The final analytic sample included 1181 women representing 2693 singleton, term births. Figure 2-1 outlines participant eligibility and exclusions.

2.3.2 Measures

Exposure: Prepregnancy weight and pregnancy weight gain amount for each live birth reported were collected by retrospective self-report at visit 13 (when women ranged in age from 56 to 68). Interviews were conducted in English, Spanish, Cantonese, and Japanese depending on site and participant. Prepregnancy BMI was calculated with the retrospective prepregnancy weight collected at visit 13 and height measured at baseline (visit 0). Adequacy of GWG, the primary

exposure, was calculated for each pregnancy based on the Institute of Medicine's 2009 guidelines [31] and characterized as "inadequate," "adequate," or "excessive" as per prepregnancy BMI category (see Appendix B, Table 1). The Institute of Medicine guidelines represent the current state of knowledge on the impact of GWG on infant and maternal health. Because these are the most clinically relevant today, we applied the 2009 recommendations in this analysis as opposed to prior standards.

Alternative BMI cutoffs were used for Japanese and Chinese participants, with overweight defined as ≥23 kg/m^2 and obese as ≥25 kg/m^2. This is consistent with recommendations from the Western Pacific Region WHO and based on literature demonstrating higher body fat in Asians at lower BMI values compared to European heritage populations.[158] Prior research supports these cut-offs for Japanese and Chinese populations living in Asia[159-162] and people of Asian heritage living in North America [163,164]. No adjustments were made to GWG adequacy cut-points as the IOM recommends these guidelines across racial/ethnic groups. Recent research has supported use of the IOM guidelines to predict maternal outcomes in Asian and Asian American populations.[165,166]

Outcome: The primary outcome was midlife BMI (kg/m^2), calculated from weight and height measured at the SWAN baseline visit by trained staff according to a standard protocol when women ranged in age from 42 to 53 years. Obesity was defined as BMI ≥ 30 kg/m^2 for Caucasian, Black, and Hispanic women and BMI ≥ 25 kg/m^2 for Japanese and Chinese women as noted above.

Covariates: Covariates collected at the baseline visit – concurrent with the outcome assessment – were age (years), race/ethnicity (Non-Hispanic Black, Chinese, Japanese, Hispanic, Non-Hispanic White), education level (categorized as high school or less, some college/college degree, or post-college study), age at first pregnancy (years), time since last pregnancy (years),

smoking status at midlife (current, previous, or never smoker), adolescent BMI (kg/m^2), difficulty in paying for basics (somewhat hard/very hard, or not very hard), menopausal status (premenopause or early perimenopause), daily caloric intake (kcal), physical activity (score), and stress level (score). Study site was also included as a covariate.

Adolescent BMI was collected by participant recall of high school weight. Menopausal status was assessed by self-report of bleeding over the 12 months prior to study visit. Caloric intake at midlife was calculated from responses to a modified Block interviewer-assisted food frequency questionnaire completed at the SWAN baseline visit [167]. The total physical activity score is derived from an adaptation of the Kaiser Permanente Health Plan Activity Survey used previously by SWAN investigators [168]. The score ranges from 1-15 and represents the sum of responses to 11 physical activity questions covering sports activities, non-sport leisure activities, and household/childcare activities. Stress is represented by a score summing the frequency of feeling overwhelmed during the past two weeks (1=Never to 5=Very Often) based on four component questions in the SWAN screener. Scores range from 4 (low stress) to 20 (high stress).

Additional reproductive history characteristics retrospectively self-reported at visit 13 were parity, number of pregnancies with a gestational hypertensive disorder, and number of pregnancies with gestational diabetes.

2.3.3 Statistical Analysis

Data transformations: The outcome measure BMI was transformed by natural log when treated continuously to account for skewed distribution.

Descriptive statistics for participant characteristics: We summarized participant characteristics for the full analytic sample and stratified by whether a woman had ever experienced

a pregnancy with excessive GWG. Categorical characteristics are presented by number and percent of women with differences evaluated by chi-square test. Continuous variables with approximately normal distributions are presented by mean and standard deviation, and compared by two-sided t tests. Adolescent and midlife BMI are presented by median and interquartile range with two-sided Wilcoxon p-values.

Modeling midlife BMI: The primary exposure was the number of pregnancies in a woman's life with excessive GWG. We used nonparametric locally-weighted scatterplot smoothing (loess) regression on number of excessive GWG pregnancies to identify the functional form of the association with midlife BMI.[169] The association between number of pregnancies with excessive GWG per woman and midlife log BMI was estimated by linear regression. A priori hypotheses of interaction between excessive GWG with parity and with race/ethnicity were tested in separate fully-adjusted models.

Unadjusted, minimally adjusted, and fully adjusted results are reported. Modelling the outcome as log-transformed BMI best fits the distribution of the data and demonstrates the attenuation of effect with the addition of covariates across models. To provide a clinically-relevant interpretation, adjusted means of log BMI were estimated for women with 1 to 3 births (n=1027) by treating number of excessive GWG pregnancies categorically. Adjusted means were back-transformed to the original scale for presentation. Bonferroni-adjusted p-values were calculated for pairwise group comparisons.

Modeling odds of midlife obesity: Odds ratios and 95% confidence intervals for obesity at midlife were estimated by logistic regression using the number of excessive GWG pregnancies as a continuous predictor. To assess the potential impact of the timing of excessive GWG across pregnancies, odds ratios and 95% confidence intervals for obesity were also estimated by logistic

regression using the GWG adequacy of each woman's last birth as a categorical predictor (among women with 1 to 3 births).

Reliability of the GWG Measure: In order to evaluate the reliability of the primary exposure variable—self-reported GWG—the difference between the GWG reported at the study baseline visit and at follow-up visit 13 were calculated for each pregnancy. Mean and standard deviation of these differences as well as Spearman correlations between the two GWG amounts are reported stratified by birth number. We tested whether the difference between the two GWG values varied by midlife obesity status with two-sample t tests. Because preliminary results indicated poorer correlation for GWG amounts reported on the 5^{th} or higher birth, sensitivity analyses were performed for the fully adjusted linear regression models of midlife BMI by excluding the n=13 women with ≥ 5 births.

All analyses were performed using SAS v. 9.4 (SAS Institute, Cary, NC, USA).

2.4 Results

Participant characteristics: In total, 985 women were excluded from the analytic sample for missing data. These participants were different from the women included in the present analysis in several ways including race/ethnicity and educational attainment (see Appendix B, Table 2).

Table 2-1 shows participant characteristics by excessive GWG status. Of the 1181 SWAN participants included, 715 (60.5%) women reported having no pregnancy with excessive GWG, and 466 (39.5%) reported one or more. Appendix B, Table 3 presents a cross tabulation of number of excessive GWG pregnancies by parity. Participants reported up to 8 births, with a mean parity of 2.3 births. Parity did not differ significantly by whether a woman had any excessive GWG

pregnancies. The mean time between the last birth and the outcome measure of midlife BMI was 15.0 ± 6.73 years.

Women who had ever experienced an excessive GWG pregnancy—compared to no excessive GWG pregnancies—were more likely to be Black and less likely to be Japanese than White. Additionally, women who had one or more excessive GWG pregnancies reported higher mean adolescent BMI, were younger at first pregnancy, were less likely to be never-smokers, and had a lower mean physical activity score at midlife in unadjusted comparisons. The mean age at the outcome time point was 46.6 ± 2.64 years. The median midlife BMI was 26.0 kg/m^2, and nearly one-third of participants had an obese BMI (32.7%). Median midlife BMI was 4.4 units higher among those who reported one or more excessive GWG pregnancies compared to those who did not ($p<0.001$). The prevalence of obesity at midlife was also higher among those with one or more excessive GWG pregnancies (47.6 %) compared to those with none (22.9%, $p<0.001$).

Modeling midlife BMI: A loess plot indicated an increase in BMI with each additional excessive GWG pregnancy (Figure 2-2). A change in slope after the first pregnancy with excessive GWG suggested a difference in the effect between none and any compared to the overall number of such pregnancies. To address this, we included a dichotomous term representing no excessive GWG pregnancies (coded as 0) versus one or more (coded as 1). This adjustment accounts for the piecewise nature of the slope.

Each excessive GWG pregnancy was associated with an increase of 0.078 log BMI units in the unadjusted model (Table 2-2). This association was attenuated to a coefficient of 0.020 log BMI units in the minimally adjusted model but remained statistically significant. Addition of the potential mediating variables—number of pregnancies with hypertensive disorder and number with gestational diabetes—did not substantially change the estimated effect size or p-value for the

main exposure. Hypertensive disorder was a significant, independent predictor of midlife BMI (p=0.001), while gestational diabetes was not (p=0.270). The interaction terms tested between excessive GWG with parity and with race/ethnicity were not significant (p > 0.7) and were not included in final models (results not shown). Potential collinearity of predictor variables was evaluated by variance inflation factor and determined not to be present (all values of VIF were < 4, results not shown).

Women with excessive GWG pregnancies had a higher midlife mean BMI compared to those with none (Table 2-3). Women with no excessive GWG had an adjusted marginal mean BMI of 25.4 kg/m^2 at midlife (95% CI=24.9, 25.9) compared to a BMI of 28.8 kg/m^2 for those reporting excessive GWG in three pregnancies (95% CI=27.3, 30.5). The adjusted mean BMI values for each increase from 1 to 3 excessive GWG pregnancies show a statistically significant monotonic relationship.

Modeling odds of midlife obesity: Each additional pregnancy with excessive GWG was associated with a 64% increase in odds of obesity at midlife (OR=1.64, 95% CI= 1.20, 2.25) in the fully adjusted model (p=0.002, see Figure 2-3). We also evaluated the relationship between GWG adequacy in the last pregnancy and odds of midlife obesity for those with 1 to 3 births (Figure 2-4). Among women with 1 birth, having excessive gestational weight gain in their pregnancy was not significantly associated with odds of obesity at midlife (p=0.719). Among women with two births, the association between excessive GWG in the second pregnancy and obesity was non-significant (p=0.185), although suggestive of increased odds with an OR of 1.78 (95% CI=0.93, 3.40). Excessive GWG in the third of three births was significantly associated with 3.5 times higher odds of obesity at midlife (OR= 3.54, 95% CI=1.37, 9.14, p=0.027) in fully adjusted models including adjustment for the GWG adequacy of the first and second pregnancies.

Reliability of GWG Measure: The mean differences and Spearman correlations of self-reported GWG amount collected at the baseline visit and again at follow-up 13 for each birth are presented in Appendix B, Table 4. The mean difference ranged from a low of 1.8 pounds for the first birth to a high of 9.0 pounds for the 6th birth. Self-report in this cohort showed moderate reliability for the first 4 births reported, with Spearman R^2 values ranging from 0.73 for the first birth to 0.57 for the fourth birth. Correlation of GWG amount per birth was significant ($p<0.001$) for the first 4 births reported, but not for the 5th birth and beyond.

The mean difference in GWG amounts reported, stratified by birth number, did not differ by midlife obesity status (Satterthwaite p-values > 0.17 per birth). However, the variability of mean difference was greater among women with an obese midlife BMI compared to those without (equality of variance F-test p-values < 0.01 per birth). Linear regression models of log-transformed BMI that excluded women with 5 or more births showed similar results to those of the primary analysis (Appendix B, Table 5). In fully adjusted models, excluding women with 5 or more births strengthened the association between the number of excessive GWG pregnancies and midlife BMI (Beta= 0.025, p= 0.017), and attenuated the effect of having any versus no excessive GWG pregnancies (Beta= 0.036, p=0.062).

2.5 Discussion

In this multi-ethnic cohort of parous women, 39.5% reported GWG that exceeded IOM recommendations in at least one pregnancy, consistent with prior observations.[33,36,37] The outcome of midlife obesity was experienced by 32.7% of women, measured at an average age of 46.6 (± 2.64). Nearly half (47.6%) of women with excessive GWG had an obese BMI at midlife, compared

to 22.9% of those who had never experienced excessive GWG. Our analysis found that each pregnancy with excessive GWG in a woman's life was associated with a 64% increase in the odds of obesity at midlife. This association did not vary by race/ethnicity and was independent of factors including parity, years since last birth, and physical activity level. Finally, results comparing the odds of obesity by GWG adequacy in a woman's last pregnancy were inconclusive. This suggests that the total number of excessive GWG pregnancies may have more influence on maternal weight than the order of GWG adequacy across pregnancies.

To the current literature, our study adds evidence of a cumulative effect of multiple pregnancies with excessive GWG on long term maternal health, independent of parity. Beyond the impact of ever versus never experiencing an excessive GWG pregnancy, each additional excessive GWG pregnancy contributed to higher mean midlife BMI. Notably, we demonstrated that this association extends to women of Japanese and Chinese ethnic backgrounds, who are rarely included in US studies of GWG.[166]

Our results are consistent with previous studies demonstrating an association between excessive GWG in a single pregnancy and increased long-term maternal weight.[43,44,154] Few studies to date have sought to incorporate weight gain over multiple pregnancies.[46,47] Cohen et al. found that the prevalence of obesity at age 40 increased with the number of excessive GWG pregnancies in a nationally-representative cohort.[46] Consistent with our study, the ordering of GWG adequacy across pregnancies did not affect the prevalence of obesity. Using the same cohort as the Cohen analysis, Abrams et al[47] estimated the impact of eliminating excessive GWG on incident obesity at a population level. Their analysis found that intervening in either the first or second pregnancy could significantly reduce the prevalence of midlife obesity among mothers.

The inter-pregnancy period is also of interest in understanding the impact of GWG across multiple pregnancies. Excessive GWG is positively correlated with increased postpartum weight retention.[117,118,170] Additionally, much of the effect of GWG on long-term maternal weight may be mediated through short term postpartum weight retention.[44] However, GWG adequacy remains an important opportunity for prevention, as the amount of weight retained postpartum depends on the amount gained during pregnancy. Further research is necessary to clarify the role of pregnancy complications. In our analysis, the number of pregnancies with hypertensive disorder was significantly associated with midlife BMI in fully adjusted models, while gestational diabetes was not. This finding should be investigated further in studies with more precise measures of pregnancy complications. In addition, the association between GWG and midlife weight may differ for women with preterm births. While we excluded women with a history of preterm birth, work by McClure et al. observed an association between excessive GWG in a single pregnancy and later maternal weight in a cohort that oversampled for small-for-gestational-age and preterm births.[39] Future research measuring GWG across multiple pregnancies including those with complications and preterm delivery would add to our understanding of the topic in a broader population of women.

Our study has several strengths. As noted, the ability to measure reproductive characteristics for all of our participants' births is unique in the literature. We were also able to capture GWG adequacy. Adequacy, which incorporates prepregnancy BMI, is a better representation of the health impacts of pregnancy weight compared to GWG amount alone. The use of adequacy also allowed us to adjust for inadequate GWG, so that adjusted models do not conflate insufficient gain with our definition of healthy gain. In addition, we had clinical measurement for the outcome of midlife BMI, rather than relying on self-reported weight and

height. Finally, our analysis leveraged a key strength of SWAN, the depth of descriptive covariates available for this cohort. The collection of high-quality measures of lifestyle characteristics including diet, physical activity, and stress enabled us to adjust for a wide range of potentially confounding characteristics.

Limitations of this study include the retrospective exposure measurement and loss to follow-up in the cohort. Our measure of GWG relied on retrospective self-report collected an average of 30 years after the last birth. Previous research suggests that women often underestimate prepregnancy weight and overestimate GWG.[128] Therefore, the prevalence of excessive GWG may be overestimated in studies relying on self-recall. Misclassification of women with adequate GWG as having excessive GWG could bias estimates toward the null. A meta-analysis by Headen et al. found moderate misclassification of GWG adequacy in studies relying on maternal recall of weight. Among studies measuring weight characteristics years after birth, mean deviation from true values was less than 1kg. However, the magnitude of error varied widely and was significantly greater among women with higher BMIs and those of minority race/ethnicity. In our data, repeated measures of self-reported GWG taken an average of 15.5 years apart showed moderate reliability, and the mean difference did not vary by obesity status. Moreover, the primary results were robust in sensitivity analyses. Smaller correlations between the two reports for participants with 5+ births may reflect increased difficulty recalling weight gain for each individual pregnancy. Despite known error, maternal recall has been used often in the literature as a practical measure to capture reproductive history. Collecting data prospectively for our hypothesis would be challenging given the long time period between exposures in pregnancy and midlife outcomes.

Secondly, the loss of participants between the baseline visit and follow-up visit 13 was differential. Women who were excluded from this analysis due to missing data were different from

included participants by several attributes. We sought to address this by adjusting for characteristics predictive of loss to follow-up such as stress, physical activity, and difficulty paying for basics.[171] However, it is possible that our estimates include selection bias due to loss to follow-up.

Our results contribute to growing evidence that excessive GWG impacts long-term maternal health. The implications of these findings are particularly relevant in light of recent research that questions the strength of association of GWG adequacy with gestational and birth outcomes.[172] Interventions to promote adequate GWG serve as maternal obesity prevention, in addition to impacts on birth and perinatal outcomes. Because over 90 percent of pregnant women receive some prenatal care in the US,[173] pregnancy can be seen as an important opportunity to protect women's long-term health.

2.6 Conclusion

In summary, our study found that each additional birth with excessive GWG was associated with an increase in mean maternal BMI at midlife. The cumulative effect we observed over multiple pregnancies implies that prevention of excessive GWG at any point in a woman's reproductive history can have an impact on long-term health. This highlights the importance of clinical counseling about healthy weight during pregnancy as an approach to obesity prevention. Studies with clinical measures of gestational weight gain should be conducted to confirm our findings. Further research could inform public health strategies for obesity prevention, as well as the clinical approach to maternal weight gain.

2.7 Tables and Figures

Table 2-1 Aim 1 Participant Characteristics

	All Women	Pregnancies with Excessive Gestational Weight Gain		p
		None	One or More	
Number of Women (%)	1181	715 (60.5)	466 (39.5)	
Sociodemographic Characteristics				
Age, mean ± SD	46.6 ± 2.64	46.9 ± 2.67	46.3 ± 2.57	<.001
Race/Ethnicity, n (%)				<.001
Black	309 (26.2)	167 (23.4)	142 (30.5)	
White	559 (47.3)	323 (45.2)	236 (50.6)	
Chinese	116 (9.8)	83 (11.6)	33 (7.1)	
Hispanic	72 (6.1)	48 (6.7)	24 (5.2)	
Japanese	125 (10.6)	94 (13.1)	31 (6.7)	
Education, n (%)				0.664
High school or less	253 (21.4)	147 (20.6)	106 (22.7)	
Some college/degree	652 (55.2)	400 (55.9)	252 (54.1)	
Post-college study	276 (23.4)	168 (23.5)	108 (23.2)	
Smoking Status, n (%)				<.001
Never smoker	724 (61.3)	471 (65.9)	253 (54.3)	
Past smoker	297 (25.1)	164 (22.9)	133 (28.5)	
Current smoker	160 (13.5)	80 (11.2)	80 (17.2)	
Adolescent BMI, median (IQR)	20.5 (19.0, 22.2)	20.2 (18.8, 21.6)	21.0 (19.4, 22.9)	<.001
Difficulty Paying for Basics, n (%)				0.340
Very or somewhat hard	414 (35.1)	243 (34)	171 (36.7)	
Not very hard	767 (64.9)	472 (66)	295 (63.3)	
Menopausal Status, n (%)				0.665
Early perimenopause	516 (43.7)	316 (44.2)	200 (42.9)	
Pre menopause	665 (56.3)	399 (55.8)	266 (57.1)	
Perceived Stress Score, mean ± SD	8.4 ± 2.90	8.4 ± 2.81	8.5 ± 3.04	0.482
Total Caloric Intake, mean ± SD	1826 ± 725.7	1816 ± 737.2	1842.6 ± 708.2	0.536
Physical Activity Score, mean ± SD	7.9 ± 1.76	8.0 ± 1.75	7.7 ± 1.76	0.009
Reproductive History				
Parity, mean ± SD	2.3 ± 1.07	2.2 ± 1.07	2.3 ± 1.07	0.127
Years Since Last Birth, mean ± SD	15.0 ± 6.73	15.2 ± 6.80	14.8 ± 6.62	0.280

Table 2-1 Continued

Age Pregnant First Time, mean ± SD	24.1 ± 5.48	24.4 ± 5.33	23.7 ± 5.68	0.017
Number of Excessive GWG Pregnancies, n (%)				–
0	715 (60.5)	715 (100)	–	
1	263 (22.3)	–	263 (56.4)	
2	138 (11.7)	–	138 (29.6)	
3 +	65 (5.5)	–	65 (13.9)	
Number of Inadequate GWG Pregnancies, n (%)				–
0	723 (61.2)	315 (44.1)	408 (87.6)	
1	216 (18.3)	170 (23.8)	46 (9.9)	
2	156 (13.2)	148 (20.7)	8 (1.7)	
3 +	86 (7.3)	82 (11.5)	4 (0.9)	
Outcome Status				
Body mass index, median (IQR)	26.0 (22.5, 31.1)	24.5 (21.8, 28.2)	29.0 (24.6, 34.6)	<.001
Obesity, n (%)	386 (32.7)	164 (22.9)	222 (47.6)	<.001

SD, standard deviation; IQR, interquartile range; BMI, body mass index; GWG, gestational weight gain.

Table 2-2 Change in Log-Transformed BMI at Midlife per Number of Pregnancies with Excessive Gestational Weight Gain

Predictor	Slope	se	p
Model 1, Unadjusted			
Number Excessive GWG Pregnancies	0.078	0.007	<.001
*Model 2, Minimally Adjusted**			
Number Excessive GWG Pregnancies	0.02	0.01	0.036
Number Inadequate GWG Pregnancies	-0.012	0.005	0.03
Any Excessive GWG Pregnancies (0/1)	0.048	0.018	0.008
Parity	0.009	0.006	0.143
Model 3, Fully Adjusted†			
Number Excessive GWG Pregnancies	0.021	0.01	0.031
Number Inadequate GWG Pregnancies	-0.011	0.005	0.042
Any Excessive GWG Pregnancies (0/1)	0.044	0.018	0.015
Parity	0.008	0.006	0.193
Number Pregnancies with Hypertensive Disorder	0.044	0.014	0.001
Number Pregnancies with Gestational Diabetes	0.02	0.019	0.27

Table 2-3 Adjusted marginal mean BMI in midlife by number of pregnancies with excessive gestational weight gain, among women with 1 to 3 births

Number Excessive GWG Pregnancies	LS Mean BMI (95% CI)*
0 (n=625)	25.35 (24.85–25.85)
1 (n=233)	26.80 (26.11–27.51)
2 (n=130)	27.46 (26.59–28.36)
3 (n=39)	28.83 (27.25–30.50)

BMI, body mass index; GWG, gestational weight gain.

*Data are least squares mean BMI from fully adjusted model. Model adjusted for number of pregnancies with inadequate GWG, parity, study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopausal status, stress score, caloric intake, physical activity score, years since last birth, age first pregnant, number of pregnancies with hypertensive disorder and number pregnancies with gestational diabetes.

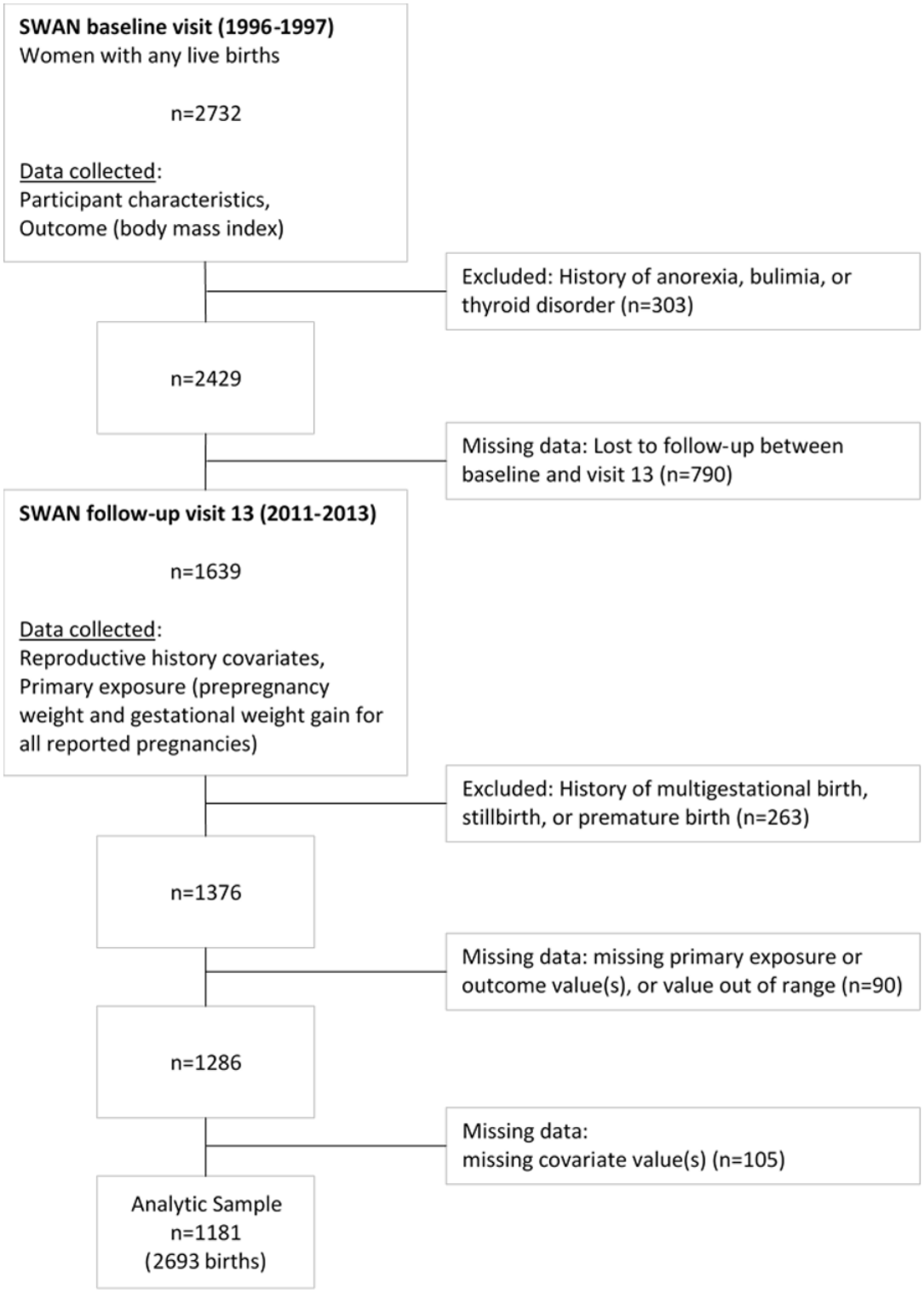

Figure 2-1 Aim 1 Participant and Data-Collection Flow-Chart

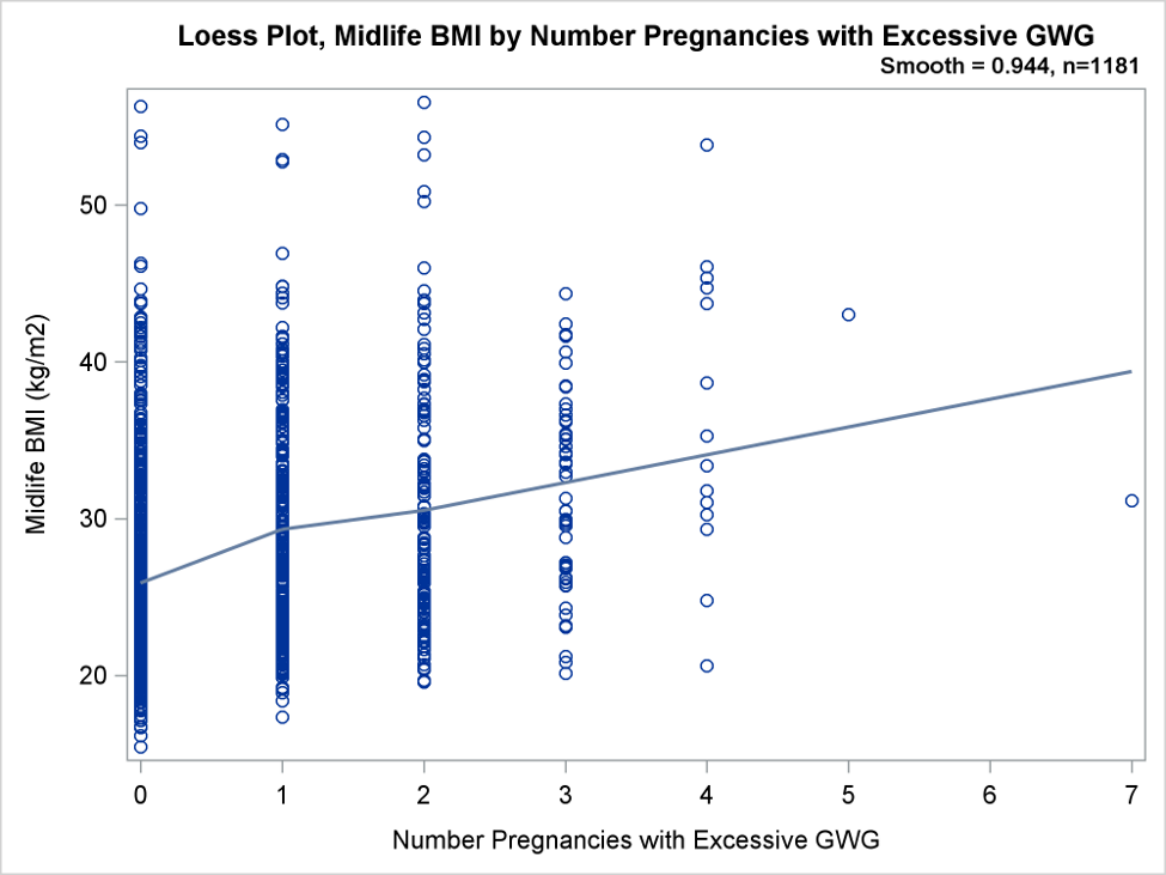

Figure 2-2 Loess Plot of Midlife BMI by Number of Excessive GWG Pregnancies

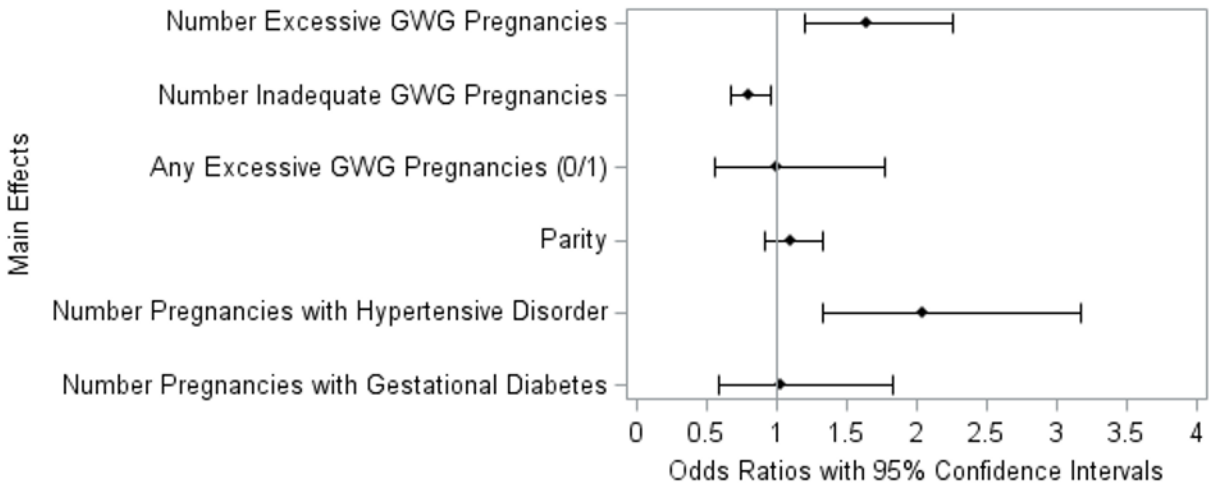

Figure 2-3 Adjusted Odds Ratios and 95% Confidence Intervals of Midlife Obesity

Figure Legend: OR, odds ratio; GWG, gestational weight gain
Model adjusted for variables shown as well as study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopausal status, stress score, caloric intake, physical activity score, years since last birth, and age first pregnant.

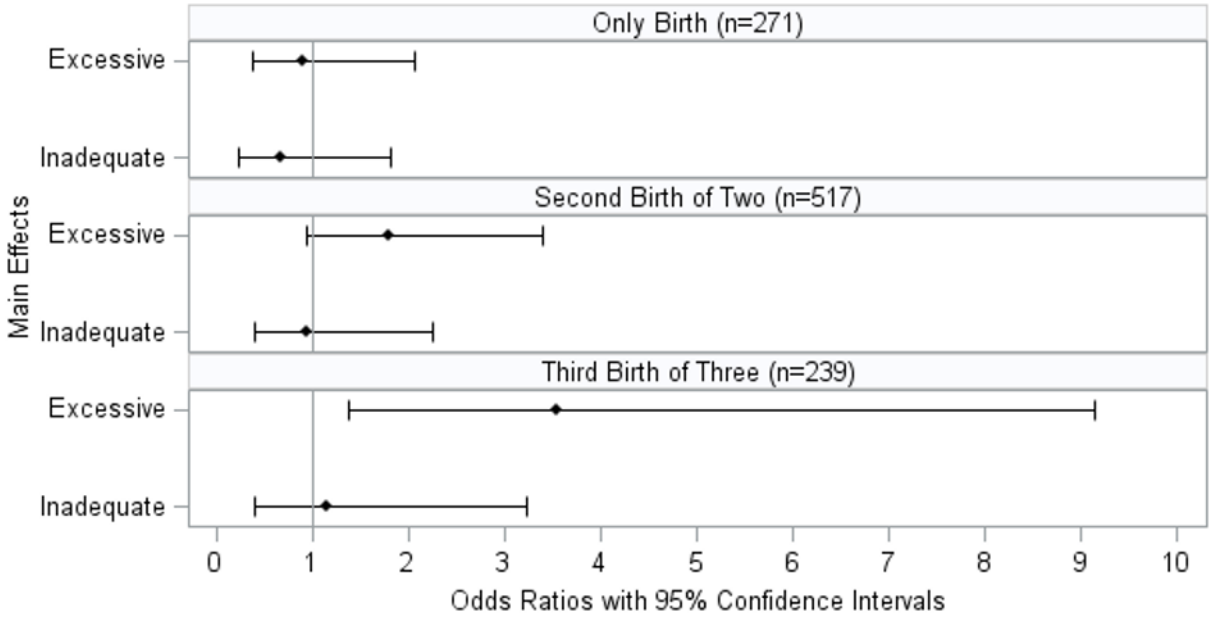

Figure 2-4 Odds Ratios and 95% Confidence Intervals of Midlife Obesity by GWG Adequacy in the Last Pregnancy, Adjusted for GWG Adequacy of Prior Pregnancies, Among Women with 1 to 3 Births

3.0 Gestational weight gain and long-term maternal obesity risk: A multiple bias analysis

Franya Hutchins[1], Robert Krafty[2], Samar R El Khoudary[1], Janet Catov[1], Alicia Colvin[1], Emma Barinas-Mitchell[1], Maria M Brooks[1].

1. University of Pittsburgh Graduate School of Public Health, Epidemiology, Pittsburgh, PA
2. University of Pittsburgh Graduate School of Public Health, Biostatistics, Pittsburgh, PA

3.1 Abstract

Background: Quantitative bias analysis is an accessible, but under-utilized, tool to address systematic error in epidemiology studies. We present a multiple-bias analytic approach to estimate the association between excessive gestational weight gain and maternal midlife obesity. Our objective was to account for selection bias due to missing data and misclassification bias due to self-report.

Methods: Participants were from the multi-ethnic Study of Women's Health Across the Nation. Obesity was defined by waist circumference measured in 1996 to 1997 when women were age 42 to 53. Gestational weight gain was measured retrospectively by self-recall and was missing for over 40% of participants. We estimated relative risk (RR) and 95% confidence intervals (CI) of obesity at midlife for presence versus absence of excessive gestational weight gain in any pregnancy. We imputed missing data via multiple imputation, and used weighted regression to account for misclassification.

Results: Among the 2,339 women in this analysis, 937 (40.1%) experienced obesity in midlife. In complete case analysis, women with any excessive gestational weight gain had an estimated 40% greater risk of obesity (RR=1.40, CI=1.13, 1.74), adjusted for confounders. Imputing data resulted in a similar estimate (RR=1.38, CI=1.16, 1.64), while RR weighted for misclassification ranged from 1.10 (0.91, 1.34) to 4.45 (3.49, 5.68) depending on the assumed sensitivity and specificity. Only models with improbable assumptions produced CIs that included a null result. Conclusions: The inference of a positive association between excessive gestational weight gain and midlife obesity is robust to methods accounting for selection and misclassification bias.

3.2 Introduction

Quantitative bias analysis is an important but under-utilized tool in life course epidemiology. To measure characteristics that develop over time, studies often require long follow-up or retrospective data collection. Both approaches are vulnerable to systematic error. This error may or may not bias estimates of association away from the true effect, and intuition around these biases is generally poor.[174,175] A range of accessible analytic options have been developed to quantify the potential bias around an estimate.[124,176]

We present a multiple-bias analysis of the association observed between excessive gestational weight gain and midlife obesity in the Study of Women's Health Across the Nation. This study is a longitudinal cohort with over twenty years of prospective follow-up. It is one of the few studies in the United States to collect data on each birth in a participant's lifetime. However, like many longitudinal studies, some measures are vulnerable to systematic error.

We identified two potential sources of systematic error in the reproductive history data: participant attrition and use of self-recall to measure pregnancy characteristics. Nearly one third of cohort participants who would otherwise be eligible for this analysis had missing data, primarily due to study dropout. Because participant attrition is often related to demographic and clinical characteristics that are associated with health outcomes,[125-127] substantial loss to follow-up may induce critical selection bias. Misclassification bias was also a concern in these data. Our primary exposure, gestational weight gain, was measured by retrospective self-report at an average of 30 years after the participants' last birth. Self-recall is a common measure for pregnancy weight characteristics[128] and moderate validity and reliability have been documented.[128-130] However, measurement error of self-recalled pregnancy weight characteristics has been observed to bias associations between gestational weight gain and birth outcomes.[177]

Having identified these challenges, our objective was to estimate the association between excessive gestational weight gain and maternal obesity at midlife in the cohort, accounting for selection and misclassification biases.

3.3 Methods

3.3.1 Participants

The Study of Women's Health Across the Nation is a prospective, multiethnic, multi-center study designed to follow women through the menopause transition. The study was conducted at seven sites: Boston, MA, Chicago, IL, Detroit, MI, Los Angeles, CA, Oakland, CA, Newark, NJ, and Pittsburgh, PA. Each site recruited women who identified as Non-Hispanic White as well as

women from one additional race or ethnic group: Non-Hispanic Black, Hispanic, Japanese, or Chinese. Enrollment began in 1996 with the following primary eligibility criteria: age 42 to 52, having at least 1 menstrual period in the previous 3 months, no exogenous hormone use in the previous 3 months, intact uterus, at least 1 ovary, and self-identification with a designated racial/ethnic group recruited by site. More information on the sampling strategy for the study has been published previously.[155] IRB approval was obtained with each site institution and written consent given by all participants.

Participants were eligible for this analysis if they reported a history of live birth(s) at the baseline interview (conducted from 1996 to 1997). A subset of these eligible women were retained through the 13th follow-up visit (conducted in 2011 to 2013). This visit included a full reproductive history questionnaire, in which participants were asked to recall prepregnancy weight and gestational age for each birth. This allowed us to calculate adequacy of gestational weight gain per birth and account for preterm births.

Women were excluded from this analysis if they reported at baseline a history of stillbirth or multifetal birth. Women with a history of underactive thyroid were also excluded due to known associations between hypothyroidism and pregnancy complications,[178,179] and because we did not know whether the reported thyroid condition was diagnosed before, during, or after pregnancies. Women were excluded for missing the outcome (midlife waist circumference), pregnancy outcome data (i.e. live birth, stillbirth, miscarriage, or abortion), later reporting a birth that occurred after the outcome assessment, or later reporting conflicting information on pregnancy outcomes.

3.3.2 Measures

Outcome: Midlife Abdominal Obesity. The primary outcome was midlife abdominal obesity based on waist circumference measured at the cohort baseline visit when women ranged in age from 42 to 53 years. Waist circumference was measured by trained staff according to a standard protocol. Abdominal obesity was defined as a waist circumference > 80 cm for Japanese and Chinese women and ≥ 88 for White, Hispanic, and Black women. All participants included in the analytic sample had a measure for waist circumference, therefore no outcome values were imputed.

Exposure: History of Excessive Gestational Weight Gain. The primary exposure was defined as ever-having a pregnancy with excessive gestational weight gain. Total gestational weight gain for each live birth was collected by retrospective self-report at visit 13, when women ranged in age from 56 to 68. Prepregnancy body mass index (BMI) was calculated with retrospective prepregnancy weight collected at visit 13 and height measured in-clinic at the baseline visit. Each pregnancy was categorized as having inadequate, adequate, or excessive gestational weight gain per the Institute of Medicine's 2009 guidelines.[31] Pregnancies reported as term births were categorized by adequacy range, and those reported as preterm were categorized by rate (see Appendix B, Table 1).

Covariates. Covariates collected at the baseline visit – concurrent with the outcome assessment – were age (years), race/ethnicity (Non-Hispanic Black, Chinese, Japanese, Hispanic, Non-Hispanic White), parity, education level (categorized as high school or less, some college/college degree, or post-college study), age at first pregnancy (years), time since last pregnancy (years), smoking status (current, previous, or never smoker), adolescent BMI (kg/m^2), difficulty in paying for basics (somewhat hard/very hard, or not very hard), menopausal status

(premenopause or early perimenopause), daily caloric intake (kcal), physical activity (score), and stress level (score). Study site was also included as a covariate.

Characteristics measured by retrospective self-report at follow-up 13 were maternal weight prior to the first pregnancy (transformed into categorical BMI, see Appendix B, Table 1), gestational age for each pregnancy, number of pregnancies with a gestational hypertensive disorder, and number of pregnancies with gestational diabetes.

Auxiliary Variables. Multiple imputation models included all analysis variables and characteristics we hypothesized to be associated with loss-to-follow up, based on previous literature.[125-127,180-186] These were: current health insurance (yes/no), current employment status (yes/no worked for pay in the last 2 weeks), language acculturation (high versus low or medium), domestic violence (yes/no report of being "Slapped, kicked, or otherwise hurt by husband/partner or someone else important to you" in the past year), very upsetting or stressful life event in the past year (yes/no), marital status (yes/no currently married), comorbidities (yes/no self-report of ever had: heart attack/MI or angina, diabetes, arthritis or osteoarthritis, high blood pressure or hypertension, high cholesterol, overactive thyroid, osteoporosis, stroke, angina, or heart attack), social support (score 0-16), depression (CES-D scale score 0-60), hostility/cynicism (score 0-13), and four quality of life scores (0-100) calculated from the 36-Item Short Form Health Survey (SF-36): physical functioning, pain, vitality, and social functioning. All auxiliary variables were measured at the baseline visit.

3.3.3 Statistical Analysis

Participant Characteristics. Participant characteristics are presented overall and stratified by missing data status. We also summarized participant characteristics among women with

complete data, stratified by whether women had reported any pregnancies with excessive gestational weight gain or none. Categorical variables are shown as number (%), continuous variables with approximately normal distribution are shown as mean and standard deviation, and those with a skewed distribution are shown by median with first and third quartile values.

Analysis Models. Exposure, outcome, and confounding characteristics were determined with the guidance of a directed acyclic graph (Appendix B, Figure 1). Based on preliminary analysis, we additionally hypothesized that preterm birth status modified the effect of gestational weight gain adequacy on maternal midlife waist circumference. The base model estimated relative risk and 95% confidence intervals of abdominal obesity for ever- versus never-having excessive gestational weight gain using generalized linear regression under the Poisson distribution with a log link.

Accounting for Missing Data. To assess which characteristics drove missingness in our data, we modeled missingness with logistic regression as a function of all auxiliary variables (listed above) as well as the analysis variables that were available for all participants: site, race/ethnicity, parity, age, waist circumference, smoking, education, difficulty paying for basics, menopause status, stress score, caloric intake, and physical activity. We structured this descriptive analysis as a backward selection regression with study site and race/ethnicity forced into the model.

We imputed missing reproductive exposures as continuous values representing the number of pregnancies with excessive gestational weight gain, inadequate gestational weight gain, hypertensive disorder of pregnancy, and gestational diabetes. Some confounder variables also had missing data and were imputed. Our imputation method was multiple imputation by chained equations (MICE). MICE is a widely used, flexible method to address missing data. Because we hypothesized an interaction between inadequate gestational weight gain and preterm birth in the

data structure, we looked for an imputation method that could accommodate non-linear relationships. Classification and regression tree (CART) algorithms have been put forward in the literature as a promising method to create imputed datasets that maintain interactions.[136,187,188] We also included a more traditional approach using predictive mean matching and logistic regression within the MICE model for comparison. Appendix B, Figure 2 illustrates the imputation process using CART and traditional MICE.

We created 10 imputed datasets for each imputation method. Diagnostics included visual inspection of trace plots to assess convergence of models. In addition, we compared the distributions of the primary imputed variables—the number of pregnancies with excessive gestational weight gain, number with inadequate gestational weight gain, and number of preterm births—between observed and imputed values. While under the missing at random assumption the distribution of imputed versus observed values may differ, distributions should be consistent conditional on the probability of being observed. Therefore, we also estimated predicted probabilities of being observed within each imputed dataset, averaged the probabilities across imputed datasets per participant, and plotted them against each imputed variable.[189]

Accounting for Misclassification. To adjust estimates for misclassification of the exposure, we first calculated misclassification weights based on published sensitivity and specificity values for gestational weight gain recall.[128,190,191] We then estimated relative risk of midlife obesity in univariate and confounder-adjusted models weighted for misclassification.[146] We checked the resulting weighted risk estimates against alternative bias adjustment methods.[144,147]

Validation studies of pregnancy weight characteristics measured by maternal recall are well summarized by Headen et al.[128] Women often underestimate pre-pregnancy weight[177] and overestimate gestational weight gain, resulting in a trend of over-reported excessive gestational

weight gain prevalence in the literature.[128] Among studies measuring pregnancy weight characteristics multiple years after birth, the mean deviation from the true value was less than 1 kg. However, the magnitude of error varied widely among women and was significantly greater among those with higher BMIs and those of minority race/ethnicity.[128] These trends have been supported in more recent validation studies.[129,192-194]

Because studies define misclassification inconsistently, with most presenting only a measure of correlation, we went to supplementary materials when available to directly calculate observed sensitivity and specificity as guideposts in creating weights. We assumed that women's self-recall of pregnancy weight was better than chance, i.e. sensitivity + specificity > 1.

McClure et al[190] assessed the validity of maternal recall of gestational weight gain adequacy among 503 women at an average follow-up of 8 years postpartum. Based on their published data, we calculated that the overall sensitivity and specificity of recalling excessive gestational weight gain in a single pregnancy was 80% and 73%, respectively. Bodnar et al[191] compared gestational weight gain adequacy based on birth certificate data, which relies on self-report of prepregnancy weight collected at delivery, with medical records in 1204 women. This validation sample was selected using a balanced design stratified by race, weight, and gestational age categories from a large birth registry sample (n=853,559). From supplementary materials we calculated sensitivity of 85% and specificity of 86% within the validation sample for reporting high gestational weight gain (defined as reporting total gestational weight gain > 80th percentile). We also applied these rates to the reported agreement in the larger birth registry sample, resulting in sensitivity of 79% and specificity of 92%. We then tested a range of sensitivity and specificity values around these benchmarks.

We further hypothesized that women with the outcome of abdominal obesity at midlife may be more likely to over-report excessive gestational weight gain than those without obesity. This misclassification scenario is of particular concern as it could induce an artifactual positive association between excessive gestational weight gain and midlife obesity. We tested 20 combinations of sensitivity and specificity stratified by outcome status. Our references for differential misclassification were again from supplementary data published by Bodnar.[191] Within the validation sample, women with an obese BMI (n=575) had sensitivity of 76% and specificity of 85% compared to those with underweight, normal, or overweight prepregnancy BMI (n=618), who had sensitivity of 94% and specificity 87%. When agreement was applied to the registry sample, women reported with lower sensitivity and higher specificity compared to the validation sample (Table 3-5).

Software. Imputation models were run using the R mice package[195] in R version 3.6.1.[196] All other analyses were run in SAS v. 9.4 (SAS Institute, Cary, NC, USA).

3.4 Results

3.4.1 Participant Characteristics

The analytic sample included 2339 women representing 5605 births. Reproductive history or covariate data were missing for 999 (42.7%) participants (Figure 3-1). Of the 999 women with missing data, 590 (59.1%) were inactive in the study by visit 13, including 71 deaths. Women with missing data were more likely to be Black or Hispanic than White, had higher mean parity (2.5 births versus 2.3 births), and were more likely to have lower educational attainment compared to

those with complete data (Table 3-1). Women with missing data were more likely to experience the outcome of midlife abdominal obesity (45.2%) compared to those with complete data (36.3%).

Among women with full data (n=1340 participants, 3097 births), 544 (40.6%) reported at least one pregnancy with excessive gestational weight gain and 151 (11.3%) reported one or more preterm births (Table 3-2). Women who had experienced any excessive gestational weight gain pregnancy(ies) were more likely to have an overweight or obese BMI prior to their first pregnancy and to have abdominal obesity in midlife compared to parous women with no excessive gestational weight gain. There was little difference in reported stress, caloric intake, and physical activity at midlife between the gestational weight gain groups.

3.4.2 Complete Case Analysis

Among women with complete data, ever-having excessive gestational weight gain was associated with a relative risk for abdominal obesity of 1.81 (1.57, 2.09) in the unadjusted model (Table 3-3). This was attenuated to 1.40 (1.13, 1.74) in the confounder-adjusted model. This model included an interaction term between inadequate gestational weight gain and preterm birth (RR: 1.18, 95% CI: 1.01, 1.37). RR estimates for the inadequate gestational weight gain pregnancies and preterm births main effects were each less than one with confidence intervals that included one. The interaction term indicates that the relative risk for women with the combination of both inadequate gestational weight gain pregnancies and preterm births (compared to no inadequate gestational weight gain pregnancies and no preterm births) is not as low as the product of the two individual effects.

3.4.3 Accounting for Missing Data

Our logistic regression to model missingness with backward variable selection indicated that study site, lower language acculturation, lower educational attainment, lack of health insurance, smoking, higher parity, lower baseline age, and lower social support were associated with increased odds of having missing data (Appendix B, Table 6).

Compared to the observed data, imputed datasets showed higher proportions of women with excessive gestational weight gain pregnancies and preterm births (Appendix B, Table 7). Diagnostic scatter plots of the number of excessive gestational weight gain pregnancies against the predicted probability of being observed per woman appeared consistent between the observed and imputed data (Appendix B, Figure 3).

Table 3-3 shows estimates of the risk of midlife obesity for the main exposures in unadjusted and confounder-adjusted models based on the observed and the imputed data. Both imputation methods resulted in consistent but diminished associations between ever-had excessive gestational weight gain and midlife abdominal obesity. Pooled estimates in confounder-adjusted models attenuated the observed RR (95% CI) of 1.40 (1.13, 1.74) to 1.31 (1.12, 1.54) using traditional MICE datasets and 1.38 (1.16, 1.64) using CART-imputed datasets. Pooled estimates were consistent with the complete case analysis in that the risk associated with excessive gestational weight gain was stronger than that of parity. However, no meaningful interaction between inadequate gestational weight gain and preterm birth was detected in the analyses of the imputed data (RR: 1.03, 95% CI: 0.96-0.97, 1.10 in both sets of imputed data). As expected, pooled estimates had improved precision over the complete case analysis.

3.4.4 Accounting for Misclassification

Of the 1340 women observed with a full reproductive history questionnaire, 269 of the 486 (55.4%) with midlife obesity reported ever-having excessive gestational weight gain, compared to 275 of the 854 (32.2%) without midlife obesity. All estimates weighted for non-differential misclassification were further from the null compared to the observed estimate (Table 3-4). At the guidepost values of sensitivity=80% and specificity=75%, the RR (95% CI) for obesity increased from 1.81 (1.57, 2.09) to 3.11 (2.60 3.72) in the univariate model and from 1.40 (1.13, 1.74) to 2.32 (1.91, 2.82) when adjusting for confounders.

Table 3-5 presents estimates of RR of obesity for those with excessive gestational weight gain compared to those without, weighted for misclassification assuming that misreporting differed by outcome status. Our starting point assumption of sensitivity=95%, specificity=85% (without obesity), sensitivity=75%, specificity=85% (with obesity), resulted in a univariate RR of 3.34 (2.76, 4.03) and confounder-adjusted RR of 2.61 (2.11, 3.22). In all combinations tested, only the confounder-adjusted model assuming 10-point lower sensitivity and 20-point higher specificity among those without midlife obesity compared to those with obesity moved the confidence interval for the RR to include a null result. Models weighted for sensitivity and specificity values based directly from published validation studies moved estimates away from the null. Figure 3-2 illustrates the results of scenarios assuming sensitivity of 75 and 85% among those without midlife obesity.

3.5 Discussion

In this multi-ethnic cohort of US women, the association between a history of excessive gestational weight gain and risk of midlife obesity persisted after accounting for selection and misclassification biases. Imputation of missing exposure data in over 40% of participants attenuated the point estimate but did not change the interpretation. Misclassification due to self-recall of the exposure was unlikely to account for the association. Most plausible scenarios to adjust for misclassification moved estimates away from the null.

Excessive gestational weight gain has been linked to maternal obesity in a number of observational studies.[39-47] Our findings provide further evidence that excessive gestational weight gain is a risk factor for long-term maternal obesity, and a stronger driver of maternal weight than parity.[40,41,46] However, the relationships between parity, prepregnancy BMI, and long-term maternal weight are not well understood.[197] Higher pre-pregnancy BMI is a risk factor for excessive gestational weight gain.[198-200] Women with higher prepregnancy BMI are more likely to experience excessive gestational weight gain and have higher postpartum BMI compared to other parous women. Our data support an association between excessive gestational weight gain and midlife obesity independent of both parity and prepregnancy BMI.[39,44]

Most relationships observed in the complete case analysis were maintained in the imputed data. Pooled estimates across the two imputation methods were similar. In addition, distributions of imputed data from both methods were comparable to observed data when plotted against the probability of being observed. One difference in the pooled imputation results compared to complete case results was an attenuation of the interaction term between inadequate gestational weight gain and preterm birth such that the interaction was no longer clinically meaningful. The

mitigation of this estimate may suggest that the observed interaction is an artifact of the missing data pattern, as the CART algorithm should represent interactions if present in the data.

Self-recall is a practical approach to collect reproductive history information. We previously reported on the reliability of gestational weight gain self-report in this cohort.[201] Repeated recall of gestational weight gain amount was moderately correlated, with poorer reliability among women in higher parity groups. Validation studies have shown a small mean difference but high variability when comparing maternal self-recall to in-person measures of pregnancy weight characteristics.[128] In our data, all non-differential misclassification scenarios tested moved the estimate away from null. This is an often assumed, but not guaranteed, phenomenon depending on patterns of confounding.[202] Misclassification scenarios reflecting higher rates of over-reporting among women with the outcome of midlife obesity (i.e. lower specificity) also moved the RR estimate away from null in most tested combinations, including the two scenarios based on published validation data. Estimates with a confidence interval that included a null result required that women with midlife obesity have better recall if they had experienced excessive gestational weight gain (sensitivity of 85% versus 75%), but much poorer recall if they had not experienced excessive gestational weight gain (specificity of 75% versus 95%), compared to their counterparts without obesity in midlife. In contrast, as the sensitivity of recall among those wihout midlife obesity increased, estimates moved away from the null.

A strength of this analysis is the comprehensive nature of the Study of Women's Health Across the Nation. We were able to draw from a wide range of descriptive variables in analytic and imputation models. A further strength was the study's rigorous data collection methods and validity of measures, including important confounders such as physical activity and diet. Our analytic outcome, waist circumference, was collected in-clinic by trained staff. Finally, the study

sample represented women from five racial/ethnic groups including those of Japanese and Chinese descent, who are underrepresented in reproductive history studies within the US.

A lack of validity research directly relevant to our data is a limitation to this analysis. Sensitivity analyses are highly dependent on the bias parameters chosen by the investigator. Results can vary widely depending on sensitivity and specificity values or distributions tested.[144] Because the accuracy of a measure in a specific cohort is unknown without internal validation data, it is important to obtain documented rates from the literature when possible. Providing parameters close to those of the measurement of interest is required to approximate the "true" association.[123]

In summary, we sought to provide an applied example of quantitative bias analysis in life course epidemiology while investigating a clinically-relevant question in reproductive health. We estimated the risk of midlife obesity associated with a history of excessive gestational weight gain, and explored the susceptibility of this estimate to common sources of statistical bias. We found that systematic error was unlikely to account for the observed association. Useful information on risk factors can be gained from observational data even in the presence of likely systematic error, as evidenced by this analysis.

3.6 Tables and Figures

Table 3-1 Aim 2 Participant Characteristics at Time of Midlife Waist Circumference Assessment

	Total	**Missing Data Status**	
	(n=2339)	Complete Case Analysis Sample (n=1340)	Missing Reproductive History or Covariate (n=999)
Age, mean (SD)	46.4 (2.67)	46.6 (2.64)	46.2 (2.70)
Race/Ethnicity, n (%)			
Black	705 (30.1)	364 (27.2)	341 (34.1)
White	999 (42.7)	628 (46.9)	371 (37.1)
Chinese	188 (8.0)	123 (9.2)	65 (6.5)
Hispanic	235 (10.0)	89 (6.6)	146 (14.6)
Japanese	212 (9.1)	136 (10.1)	76 (7.6)
Education, n (%)			
High school or less	645 (27.9)	298 (22.2)	347 (35.6)
Some college or degree	1239 (53.5)	745 (55.6)	494 (50.7)
Post-college study	431 (18.6)	297 (22.2)	134 (13.7)
Smoking Status, n (%)			
Never smoker	1358 (58.1)	823 (61.4)	535 (53.7)
Past smoker	544 (23.3)	335 (25.0)	209 (21.0)
Current smoker	435 (18.6)	182 (13.6)	253 (25.4)
Adolescent BMI, median (Q1, Q3)	20.5 (19, 22)	20.4 (19, 22)	20.5 (19, 23)
Difficulty Paying for Basics, n (%)			
Not very hard	1332 (57.3)	846 (63.1)	486 (49.4)
Somewhat or very hard	991 (42.7)	494 (36.9)	497 (50.6)
Menopausal Status, n (%)			
Pre menopause	1261 (54.3)	745 (55.6)	516 (52.6)
Early perimenopause	1061 (45.7)	595 (44.4)	466 (47.5)
Perceived Stress Score, mean (SD)	8.6 (2.97)	8.5 (2.92)	8.9 (3.04)
Total Caloric Intake, mean (SD)	1870 (778.6)	1834 (739.9)	1920 (826.0)
Physical Activity Score, mean (SD)	7.7 (1.77)	7.8 (1.76)	7.5 (1.77)
Parity, mean (SD)	2.4 (1.13)	2.3 (1.08)	2.5 (1.19)
Years Since Last Birth, mean (SD)	15.1 (6.72)	15.0 (6.77)	15.2 (6.66)
Age Pregnant First Time, mean (SD)	23.4 (5.48)	24.0 (5.46)	22.6 (5.42)
Waist circumference (cm), median (Q1, Q3)	83.0 (74, 95)	81.3 (73, 93)	85.0 (75, 97)
Abdominal obesity, n (%)	937 (40.1)	486 (36.3)	451 (45.2)

Table 3-2 Participant Characteristics Among Those with Observed Reproductive History

	Total	Pregnancies with Excessive GWG	
	(n=1340)	None (n=796)	One or More (n=544)
Age, mean (SD)	46.6 (2.64)	46.8 (2.67)	46.2 (2.55)
Race/Ethnicity, n (%)			
Black	364 (27.2)	199 (25.0)	165 (30.3)
White	628 (46.9)	355 (44.6)	273 (50.2)
Chinese	123 (9.2)	87 (10.9)	36 (6.6)
Hispanic	89 (6.6)	54 (6.8)	35 (6.4)
Japanese	136 (10.1)	101 (12.7)	35 (6.4)
Education, n (%)			
High school or less	298 (22.2)	168 (21.1)	130 (23.9)
Some college or degree	745 (55.6)	449 (56.4)	296 (54.4)
Post-college study	297 (22.2)	179 (22.5)	118 (21.7)
Smoking Status, n (%)			
Never smoker	823 (61.4)	525 (66.0)	298 (54.8)
Past smoker	335 (25.0)	174 (21.9)	161 (29.6)
Current smoker	182 (13.6)	97 (12.2)	85 (15.6)
Adolescent BMI, median (Q1, Q3)	20.4 (18.9, 22.2)	20.2 (18.8, 21.6)	20.9 (19.2, 22.8)
Difficulty Paying for Basics, n (%)			
Not very hard	846 (63.1)	508 (63.8)	338 (62.1)
Somewhat or very hard	494 (36.9)	288 (36.2)	206 (37.9)
Menopausal Status, n (%)			
Pre menopause	745 (55.6)	435 (54.6)	310 (57.0)
Early perimenopause	595 (44.4)	361 (45.4)	234 (43.0)
Perceived Stress Score, mean (SD)	8.5 (2.92)	8.4 (2.86)	8.5 (3.02)
Total Caloric Intake, mean (SD)	1834 (739.9)	1830 (752.1)	1841 (722.4)
Physical Activity Score, mean (SD)	7.8 (1.76)	7.9 (1.77)	7.7 (1.74)
Parity, mean (SD)	2.3 (1.08)	2.3 (1.09)	2.4 (1.05)
Years Since Last Birth, mean (SD)	15.0 (6.77)	15.3 (6.89)	14.5 (6.56)
Age Pregnant First Time, mean (SD)	24.0 (5.46)	24.2 (5.42)	23.5 (5.5)
Number of Excessive GWG Pregnancies, n (%)			
0	796 (59.4)	796 (100)	0 (0)
1	312 (23.3)	0 (0)	312 (57.4)
2	156 (11.6)	0 (0)	156 (28.7)
3 +	76 (5.7)	0 (0)	76 (14.0)
Any Preterm Birth, n (%)	151 (11.3)	77 (9.7)	74 (13.6)
BMI Category Prior to First Birth			
Underweight	171 (12.8)	115 (14.5)	56 (10.3)
Normal weight BMI	989 (73.8)	620 (77.9)	369 (67.8)
Overweight	125 (9.3)	43 (5.4)	82 (15.1)
Obese	55 (4.1)	18 (2.3)	37 (6.8)
Waist circumference (cm), median (Q1, Q3)	81.3 (73.4, 93.0)	78.4 (71.7, 87.5)	87.1 (77.5, 100.2)
Abdominal obesity, n (%)	486 (36.3)	217 (27.3)	269 (49.4)

Table 3-3 Complete Case and Pooled Regression Estimates: Relative Risk of Midlife Obesity

Predictor	Complete Case (n=1340)		Pooled Traditional MICE (n=2339)		Pooled CART (n=2339)	
	RR	95% CI	RR	95% CI	RR	95% CI
Model 1: Unadjusted						
Ever had excessive GWG [a]	1.81	(1.57, 2.09)	1.67	(1.50, 1.86)	1.68	(1.50, 1.87)
Model 2: Adjusted [b]						
Ever had excessive GWG	1.40	(1.13, 1.74)	1.31	(1.12, 1.54)	1.38	(1.16, 1.64)
Parity	1.07	(0.97, 1.18)	1.08	(1.01, 1.16)	1.07	(1.00, 1.15)
Inadequate GWG pregnancies	0.93	(0.82, 1.04)	0.96	(0.88, 1.06)	0.98	(0.90, 1.06)
Gestational hypertension	1.13	(0.92, 1.39)	1.06	(0.91, 1.23)	1.10	(0.94, 1.29)
Gestational Diabetes	1.20	(0.87, 1.64)	1.11	(0.90, 1.36)	1.01	(0.79, 1.29)
Preterm Births	0.79	(0.59, 1.07)	0.94	(0.78, 1.12)	0.94	(0.78, 1.13)
Inadequate GWG*Preterm Birth	1.18	(1.01, 1.37)	1.03	(0.96, 1.10)	1.03	(0.97, 1.10)

Footnotes:

[a] Abbreviations: CI, confidence interval; GWG, gestational weight gain; RR, relative risk.

[b] Model 2 adjusted for variables shown as well as study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopause status, stress score, caloric intake, physical activity score, years since last birth, age first pregnant, and BMI category prior to the first pregnancy.

Table 3-4 Misclassification-Adjusted RR (95% CI) of Midlife Abdominal Obesity for Ever-had Versus No Excessive Gestational Weight Gain

Sensitivity (%)	Specificity (%)	Univariate RR (95% CI)	Confounder-Adjusted [a] RR (95% CI)
75	75	3.41 (2.84, 4.09)	2.55 (2.10, 3.11)
75	85	2.76 (2.28, 3.34)	2.14 (1.74, 2.63)
75 [b]	**95**	2.48 (2.03, 3.02)	1.95 (1.57, 2.42)
80	**75**	3.11 (2.60 3.72)	2.32 (1.91, 2.82)
80	85	2.52 (2.10, 3.03)	1.95 (1.60, 2.39)
80	95	2.26 (1.87, 2.73)	1.78 (1.44, 2.19)
85	75	2.91 (2.44, 3.48)	2.16 (1.78, 2.63)
85	**85**	2.36 (1.97, 2.83)	1.82 (1.49, 2.23)
85	95	2.12 (1.76, 2.55)	1.66 (1.35, 2.05)
90	75	2.77 (2.32, 3.32)	2.05 (1.68, 2.49)
90	85	2.25 (1.88, 2.69)	1.73 (1.42, 2.11)
90	95	2.02 (1.68, 2.42)	1.58 (1.28, 1.94)

Footnotes:

[a] Confounder-adjusted model includes number inadequate gestational weight gain pregnancies, parity, number pregnancies with hypertensive disorder, number pregnancies with gestational diabetes, preterm birth, an interaction term for inadequate gestational weight gain by preterm birth, study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopause status, stress score, caloric intake, physical activity score, years since last birth, age first pregnant, and BMI category prior to the first pregnancy.

[b] Bold font indicates sensitivity and specificity values estimated from previous validation studies.

Table 3-5 Misclassification-Adjusted RR (95% CI) of Midlife Obesity for Ever-had Versus No Excessive Gestational Weight Gain, Assuming Differential Misreporting by Outcome Status

Those without obesity		Those with obesity			
Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Univariate RR (95% CI)	Confounder-Adjusted [a] RR (95% CI)
75	85	65	85	4.61 (3.68, 5.78)	3.59 (2.83, 4.56)
75	85	65	75	3.72 (3.02, 4.58)	2.88 (2.31, 3.59)
75	95	65	75	2.87 (2.33, 3.53)	2.25 (1.81, 2.81)
75	85	75	85	2.76 (2.28, 3.34)	2.14 (1.74, 2.63)
75	95	75	75	1.76 (1.46, 2.11)	1.41 (1.16, 1.71)
75	85	85	85	2.11 (1.76, 2.53)	1.64 (1.35, 2.00)
75	85	85	75	1.77 (1.48, 2.12)	1.40 (1.16, 1.69)
75	95	85	75	1.35 (1.13, 1.61)	1.10 (0.91, 1.34)
85 [b]	**90**	**65**	**90**	4.94 (3.91, 6.24)	3.94 (3.07, 5.06)
85	95	65	75	3.25 (2.64, 4.00)	2.56 (2.05, 3.20)
85	85	75	85	3.07 (2.54, 3.71)	2.39 (1.94, 2.94)
85	95	75	75	1.99 (1.66, 2.39)	1.58 (1.29, 1.92)
85	85	85	85	2.36 (1.97, 2.83)	1.82 (1.49, 2.23)
85	95	85	75	1.53 (1.28, 1.83)	1.23 (1.01, 1.49)
95	85	65	85	5.55 (4.43, 6.95)	4.45 (3.49, 5.68)
95	95	65	75	3.58 (2.91, 4.40)	2.85 (2.27, 3.57)
95	**85**	**75**	**85**	3.34 (2.76, 4.03)	2.61 (2.11, 3.22)
95	95	75	75	2.20 (1.83, 2.64)	1.73 (1.42, 2.11)
95	85	85	85	2.57 (2.15, 3.08)	1.99 (1.62, 2.43)
95	95	85	75	1.70 (1.42, 2.03)	1.34 (1.10, 1.63)

Footnotes:

[a] Confounder-adjusted model includes number inadequate gestational weight gain pregnancies, parity, number pregnancies with hypertensive disorder, number pregnancies with gestational diabetes, preterm birth, an interaction term for inadequate gestational weight gain by preterm birth, study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopause status, stress score, caloric intake, physical activity score, years since last birth, age first pregnant, and BMI category prior to the first pregnancy.

[b] Bold font indicates sensitivity and specificity values estimated from previous validation studies.

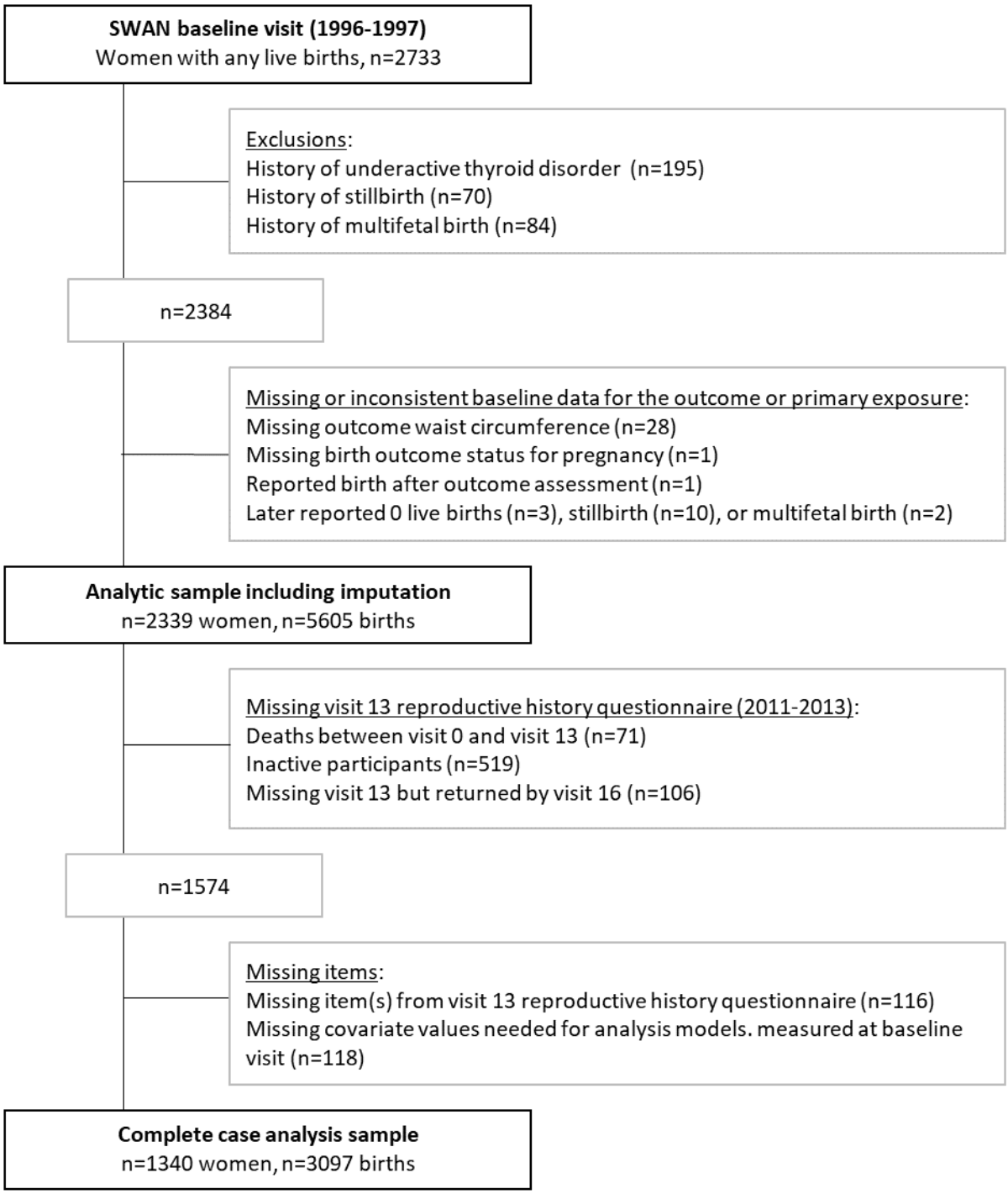

Figure 3-1 Aim 2 Participant Flow-Chart

Figure legend: Figure illustrates participant eligibility and missing data for selecting the analytic sample from the Study of Women's Health Across the Nation.

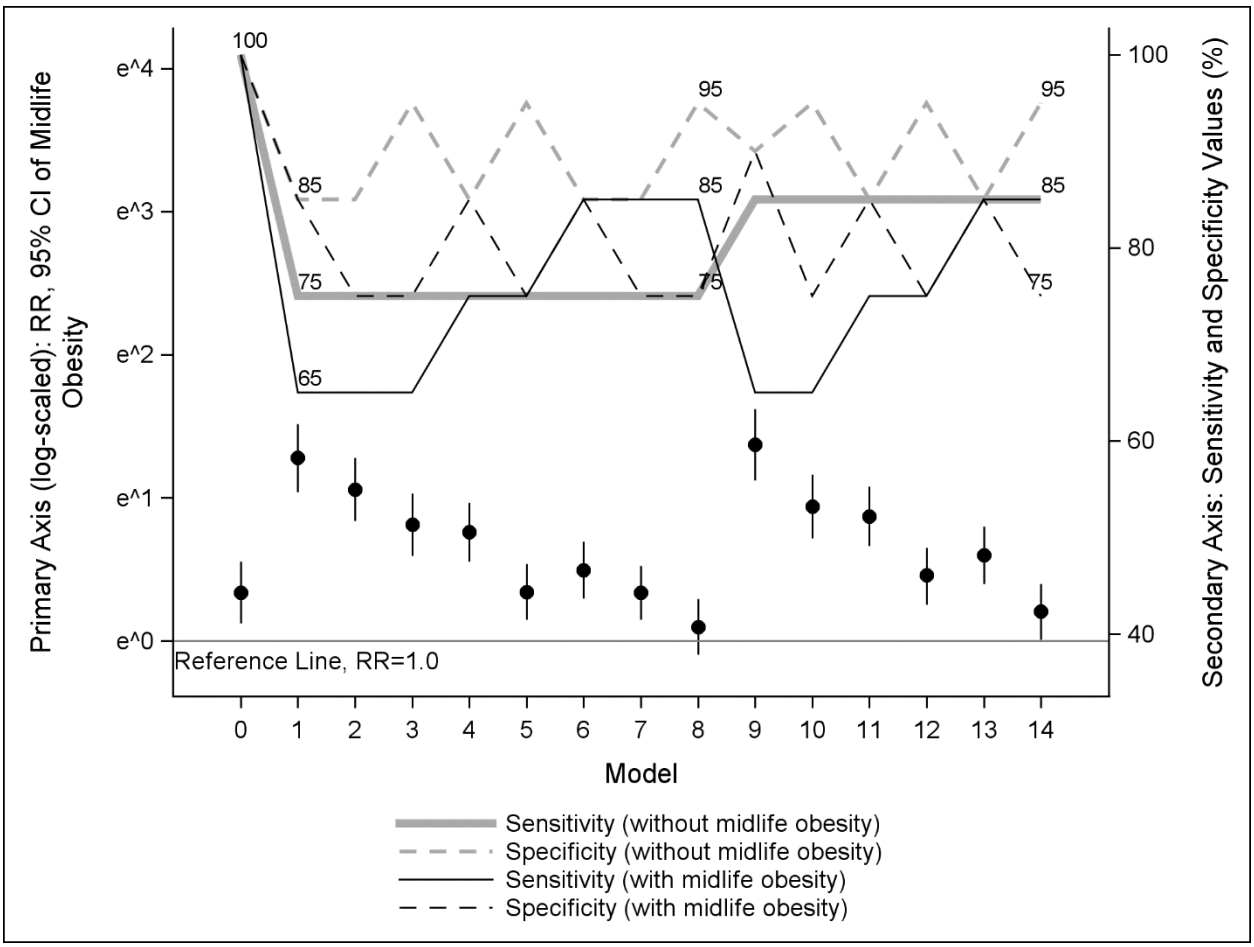

Figure 3-2 Relative Risk of Midlife Obesity for Ever- versus Never-Had Excessive Gestational Weight Gain, Confounder-Adjusted, by Sensitivity and Specificity of Self-Report

Figure Legend: Primary axis shows relative risk of midlife obesity with 95% confidence intervals. Secondary axis shows the sensitivity and specificity values assumed for each model, which are stratified by midlife obesity status. Model 0 represents the observed estimate (i.e. sensitivity=100%, specificity=100%). All models adjust for the following confounders: number inadequate gestational weight gain pregnancies, parity, number pregnancies with hypertensive disorder, number pregnancies with gestational diabetes, preterm birth, interaction term for inadequate gestational weight gain by preterm birth, study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopause status, stress score, caloric intake, physical activity score, years since last birth, age first pregnant, and BMI category prior to the first pregnancy.

4.0 Excessive Gestational Weight Gain and Long-Term Maternal Cardiovascular Risk: Two Decades of Follow-up in the Study of Women's Health Across the Nation

Franya Hutchins[1], Samar R El Khoudary[1], Janet Catov[1], Robert Krafty[2], Alicia Colvin[1], Emma Barinas-Mitchell[1], Maria M Brooks[1].

1. University of Pittsburgh Graduate School of Public Health, Epidemiology, Pittsburgh, PA
2. University of Pittsburgh Graduate School of Public Health, Biostatistics, Pittsburgh, PA

4.1 Abstract

Background: Excessive gestational weight gain (GWG) is consistently linked with long-term maternal risk of obesity. However, the literature on GWG and cardiovascular risk factors such as dyslipidemia, diabetes, and chronic inflammation is minimal and conflicting. We sought to evaluate whether a history of excessive GWG contributes to atherosclerotic cardiovascular risk among parous women in midlife.

Methods: Data were from the multi-ethnic cohort Study of Women's Health Across the Nation. Excessive GWG was collected by self-recall of pregnancy weight and defined according to Institute of Medicine guidelines. Outcomes were the atherosclerotic cardiovascular disease (ASCVD) risk score and C-reactive protein (CRP), measured at 11 study visits conducted from

1996 to 2017. Our primary analysis estimated the effect of excessive GWG on log-transformed ASCVD score and CRP via linear mixed model regression.

Results: The analytic sample was comprised of 1318 women with 3049 births. Over 40% (536) reported one or more pregnancies with excessive GWG. In unadjusted longitudinal models, a history of excessive GWG was associated with a 29.6% increase in ASCVD risk score (95% CI=18.2, 42.1) and 89.2% (63.2, 119.4) increase in CRP. Associations were attenuated to a 16.1% (6.8, 26.2) increase in ASCVD score and 49.9% (29.9, 73.1) in CRP with the addition of confounders including parity, demographic characteristics, and physical activity. Confounder-adjusted models estimated a mean 10-year ASCVD risk of 9.8% (9.2, 10.5) versus 9.5% (8.9, 10.1) and mean CRP of 2.20 mg/l (1.89, 2.57) versus 1.85 mg/l (1.61, 2,14) at 20 years of midlife follow-up for those with and without excessive GWG, respectively.

Conclusions: In this multi-ethnic cohort of parous women, a history of excessive GWG was associated with a small but statistically significant increase in atherosclerotic CVD risk, and a moderate, statistically significant increase in CRP across midlife. More research is necessary to understand the mechanisms underlying the pathway between excessive GWG and long-term maternal cardiovascular health.

4.2 Introduction

Obesity is a highly prevalent[13] risk-enhancing factor for cardiovascular disease.[203] In the United States, women in their midlife experience the highest rates of obesity.[13] An opportunity to prevent obesity ahead of midlife is during pregnancy. Excessive gestational weight gain is common[33,37] and has consistently been linked with increased long-term maternal

weight.[39,40,43,44,46,170] Prevention of excessive gestational weight gain could lead to population-level declines in obesity rates.[47]

Despite the link between obesity and cardiovascular disease, research has not consistently observed an association between excessive gestational weight gain and increased cardiovascular risk.[39,42,48] This could be due to difficulty in implementing studies with sufficient follow-up to observe reproductive characteristics and long-term cardiovascular outcomes. Alternatively, excessive gestational weight gain may contribute to a low-risk phenotype of obesity. Research increasingly supports a view of obesity as a heterogeneous characteristic.[18,19,23,25] The risk conveyed by obesity appears to vary by metabolic function,[17] a relationship that is not well understood and may change over time.[21,22,204] In addition to traditional cardiovascular risk factors, inflammation is gaining interest as a key part of the atherosclerotic pathway.[205-207] Due to its relationship with metabolic function, inflammation may be part of the mechanism linking obesity and cardiovascular risk.[208-211] No study to our knowledge has sought to evaluate the hypothetical pathway of excessive gestational weight gain through maternal obesity and long-term chronic inflammation.

The objective of this study is to evaluate the long-term impact of excessive gestational weight gain on cardiovascular risk in the multi-ethnic cohort Study of Women's Health Across the Nation (SWAN). The SWAN study has been an important source of data on women's health through midlife and provides over 20 years of prospective follow-up.[148,149,212]

We use the atherosclerotic cardiovascular disease (ASCVD) risk score and the inflammatory biomarker C-reactive protein (CRP) to describe participants' cardiovascular risk profile. The ASCVD score is a clinically relevant and interpretable representation of the most recent literature on the effect of traditional CVD risk factors. Using C-reactive protein as a

secondary outcome, our analysis captures dyslipidemia, hypertension, diabetes, and inflammation. Specifically, we hypothesized that a history of excessive gestational weight gain would be associated with increased ASCVD risk score and CRP among parous women as they transition through midlife into early old age.

4.3 Methods

4.3.1 Participants

SWAN is an ongoing, prospective cohort study designed to observe health characteristics in women through the menopause transition. The SWAN cohort is made up of 3302 women enrolled from seven cities: Boston, MA, Chicago, IL, Detroit, MI, Los Angeles, CA, Oakland, CA, Newark, NJ, and Pittsburgh, PA. Enrollment began in 1996 with the following primary eligibility criteria: age 42 to 52, having at least one menstrual period in the previous 3 months, no exogenous hormone use in the previous three months, intact uterus, at least one ovary, and self-identification with a designated racial/ethnic group recruited by site. All sites enrolled women who identified as Non-Hispanic White as well as women from one additional race or ethnic group: Non-Hispanic Black, Hispanic, Japanese, or Chinese. Information on the sampling strategy for SWAN has been published previously.[212] IRB approval was obtained with each site institution and written consent given by all participants. The SWAN study includes a baseline visit (conducted from 1996 to 1997), and 16 follow-up visits.

Figure 4-1 illustrates the eligibility and exclusions of the participant sample for this analysis. At the baseline visit, 2733 SWAN participants reported a history of live birth(s). Women

were excluded if they reported history of underactive thyroid disorder (n=195), current hormone use (n=6), or history of stillbirth or multifetal birth (n=154) at baseline. Women with missing waist circumference, ASCVD score, or C-reactive protein measure at baseline were excluded (n=78). An additional 17 women were excluded for reporting conflicting birth information during follow-up or reporting a birth after the baseline visit. The remaining 2283 women were considered eligible for the analysis.

A subset of these eligible women were retained through the 13th follow-up visit (conducted in 2011 to 2013), which included a comprehensive reproductive history questionnaire. This questionnaire collected pre-pregnancy weight and gestational age for each birth, which are necessary to calculate gestational weight gain adequacy. Women who are missing the reproductive history questionnaire (n=750), questionnaire items, or covariate values measured at baseline (n=215) were excluded from the complete case sample but included in the imputed data sample (see below). The final analytic sample was 1318 women with 3049 births in complete case analyses, and 2283 women with 5475 births in imputed data analyses.

4.3.2 Measures

Outcomes: The outcomes in this analysis are the ASCVD risk score and C-reactive protein. Each was collected at baseline and at 10 of the 16 SWAN follow-up visits, representing 20 years of prospective follow-up.

The ASCVD risk score measures the 10-year risk of developing a first atherosclerotic cardiovascular disease event. Events include nonfatal myocardial infarction, coronary heart disease death or fatal or nonfatal stroke. The score was developed by a working group of the American Heart Association and the American College of Cardiology, and is calculated using the

following components: use of blood pressure medications, current smoking status, current diabetes status, and log-transformed values of age, total cholesterol, high density lipoprotein (HDL) cholesterol, and systolic blood pressure (measures described below). Each component is multiplied by a race-specific coefficient, summed for a total score, and transformed by an overall race-specific formula.[5] The ASCVD score is available for SWAN baseline and follow-up visits 1, 3-7, 9, 12, 13, and 15. It was set to missing if any component values were missing.

ASCVD score components were measured as follows: The use of blood pressure medications was ascertained through medication data collected during the interview and also at the time of specimen collection (baseline through visit 10), or via a separate worksheet (visits 12-15). The medication data were reviewed, identified, and coded by SWAN Coordinating Center personnel. Medications were then coded to therapeutic class(es) according to the Iowa Drug Information System (IDIS) database. Participants were considered to have diabetes if they met any of the following criteria: A. Use of anti-diabetic medication at any visit; B. Had a fasting glucose ≥126 (while not on steroids) at 2 consecutive visits or on 50% of at least 3 attended visits; or, C. Had two visits with self-reported diabetes and at least one visit with fasting glucose ≥126 (while not on steroids). Total and HDL cholesterol were analyzed in serum and plasma from fasting blood samples obtained at baseline and follow-up visits 1, 3-7, 9, and 12-15. Samples were analyzed at the Medical Research Laboratory (MRL) of Lexington, Kentucky or the University of Michigan Pathology laboratory (Ann Arbor, Michigan) depending on visit. Lipid values were calibrated to be comparable across changes in lab. Blood pressure is represented by the mean of two systolic blood pressure measurements. Blood pressure measures were taken by trained staff after 5 minutes quiet sitting with two minutes between measures. Smoking status was obtained by self-report.

CRP was measured at baseline and visits 1, 3-7, 9, 10, 12 and 15. Blood samples were obtained under fasting conditions (no food or drink except water in the previous 12 hours). CRP was analyzed in plasma. Samples obtained at the baseline visit through follow-up visit 7 were analyzed at the MRL by immunonephelometry using Behring reagents on the Behring Nephelometer II. Samples obtained at visits 9, 10, and 15 were analyzed at the University of Michigan Central Ligand Assay Satellite Services (CLASS) laboratory of Ann Arbor, MI on the Alfa-Wasserman ACE analyzer with the ACE hs-CRP assay. Visit 12 samples were analyzed at the CLASS laboratory using a latex-enhanced turbidimetric *in vitro* immunoassay on the Alfa-Wasserman ACE analyzer via ACE CRP *Ultra* Wide Range Reagent Kit. A calibration equation was developed by the SWAN Coordinating Center using randomly sampled (n=200) calibration samples across the full range of values for each assay method for a total of 600 samples. The calibration was developed and applied to convert the MRL and CLASS assays to a high sensitivity assay (ELISA). The Human High Sensitivity CRP ELISA (R&D Systems, DCRP00) is a plate assay which employs the quantitative sandwich enzyme immunoassay technique using a monoclonal antibody specific for CRP. To obtain values from the original assays that were below the lower limit of detection (LLD), the ELISA assay was run on all samples where the values were below the original LLD.

Primary Exposure: The primary exposure was a history of excessive gestational weight gain. Total gestational weight gain for each live birth was collected by retrospective self-report at visit 13, when women ranged in age from 56 to 68. Pre-pregnancy body mass index was calculated with retrospective pre-pregnancy weight collected at visit 13 and height measured in-clinic at the baseline visit. Each pregnancy was categorized as having inadequate, adequate, or excessive gestational weight gain per the Institute of Medicine's 2009 guidelines.[31] These guidelines provide

a range of gestational weight gain amount considered clinically "adequate" depending on maternal pre-pregnancy BMI (see Table 4-1). Women who reported any pregnancy with gestational weight gain above the adequacy range for their pre-pregnancy BMI and gestational age at birth were categorized as having a history of excessive gestational weight gain.

In creating the categorical gestational weight gain adequacy variables, alternative BMI cutoffs were used for Japanese and Chinese participants, with overweight defined as ≥23 kg/m^2 and obese as ≥25 kg/m^2. This is consistent with recommendations from the World Health Organization's Western Pacific Regional Office[213] and prior research in Japanese and Chinese populations living in North America.[163,164]

Covariates: Covariates collected at the SWAN baseline visit when women ranged in age from 42 to 53 years were race/ethnicity (Non-Hispanic Black, Chinese, Japanese, Hispanic, Non-Hispanic White), study site, education (high school or less, some college/college degree, or post-college study), daily caloric intake (calculated from responses to a modified Block interviewer-assisted food frequency questionnaire), age first pregnant (years), time since the last birth (years), parity, and smoking status (ever or never smoker). Abdominal obesity at the baseline visit was considered a potential mediator in this analysis and was based on waist circumference measured in clinic, defined as > 80 cm for Japanese and Chinese women and ≥ 88 for Black, Hispanic, and White women.

Characteristics measured retrospectively at the follow-up 13 visit were: maternal BMI category prior to the first pregnancy defined using self-recalled weight (see Table 4-1), history of excessive gestational weight gain, inadequate gestational weight gain, preterm birth defined using gestational age for each birth, hypertensive disorder of pregnancy, and gestational diabetes.

Time varying covariates were age (years), menopause status, current hormone use, difficulty paying for basics (somewhat hard/very hard, or not very hard), stress score, physical activity score, and current statin use (yes/no). Menopause status was defined in SWAN by the follow 7 categories: premenopausal (no change in bleeding patterns), early perimenopause (change in length bleeding pattern), late perimenopause (no bleeding in 3-11 months), natural postmenopause (no bleeding in 12 months not due to hysterectomy), surgical menopause (bilateral oophorectomy with or without hysterectomy), hysterectomy with one or two ovaries retained, and hormone use before final menstrual period. The stress score range is 4 (low stress) to 20 (high stress) and is calculated as the sum of the four component questions regarding frequency of feeling overwhelmed during the past two weeks, defined as 1=Never to 5=Very Often. The physical activity score range is 1 to 15 and is derived from an adaptation of the Kaiser Permanente Health Plan Activity Survey (used previously in SWAN).[168] Some visits did not collect information on difficulty paying for basics or physical activity. Values in those visits are carried forward from the visit prior.

4.3.3 Statistical Analysis

Descriptive: Participant characteristics at baseline are presented overall and stratified by excessive gestational weight gain history. Categorical variables are shown as number with percent, and continuous variables by mean with standard deviation if normally distributed or median with first and third quartile values if skewed.

Analysis Models: ASCVD score and CRP level were transformed by natural log due to skewed distributions. We modeled baseline log-transformed ASCVD score and CRP, separately, as functions of excessive gestational weight gain history using linear regression. Regression

coefficients and 95% confidence intervals are shown as percent change using the transformation $100(e^{\beta}-1)$. We present unadjusted estimates, those adjusted for confounders (race/ethnicity, site, age, difficulty paying for basics, education level, caloric intake, physical activity, stress score, smoking history, BMI prior to first pregnancy, age first pregnant, years between last birth and baseline visit, parity, history of inadequate gestational weight gain, and menopause status), and estimates adjusted for confounders as well as characteristics we hypothesized to be potential mediators (preterm birth, pregnancy complications, and midlife obesity). Adjusted models of CRP also include statin use as a confounder.

We then estimated level and change over time of ASCVD score and CRP using longitudinal mixed-effect regression models. Outcomes were modeled as a function of excessive gestational weight gain history, participant age at baseline, time since the baseline visit, and an interaction term for excessive gestational weight gain by time. The intercept and time were set as random effects. Covariate adjustment included the variables described above, with menopause status, hormone use, difficulty paying for basics, stress, physical activity, and statin use included as time-varying. We estimated least squares means of each outcome by excessive gestational weight gain status, back-transformed to the original scale for interpretation. To accommodate possible change in slope over time, least squares means were estimated with follow-up set to three separate time points: 0, 10, and 20 years after SWAN baseline.

We chose not to exclude participants with high CRP levels even though this might reflect acute infection. Recent evidence indicates that high-risk individuals such as those with a high BMI may maintain CRP levels above 10 mg/L over multiple years, implying that commonly used cut-off values are too conservative to capture the full range of chronic inflammation.[214]

Sensitivity Analysis: To evaluate the susceptibility of our estimates to bias due to missing data, we conducted a sensitivity analysis of baseline estimates with imputed data representing the full eligible sample. Missing reproductive history and covariate values were imputed using multiple imputation by chained equations with classification and regression trees as the internal prediction algorithm.[136,188] We created 10 imputed datasets and pooled estimates of the confounder-adjusted effect of excessive gestational weight gain on each outcome.

Software: Imputation models were run using the R mice package[195] in R version 3.6.1[196]. All other analyses were run in SAS v. 9.4 (SAS Institute, Cary, NC, USA).

4.4 Results

Descriptive: Of the 2283 eligible participants, 965 were excluded for missing reproductive history data or covariates. The women with missing data were systematically different from complete cases in a number of ways including race/ethnicity and educational attainment (see Appendix B, Table 8). Women with missing data also had higher median ASCVD scores (0.11% versus 0.08% 10-year risk) and higher median CRP (2.36 versus 1.48 mg/L) at baseline compared to women in the complete case sample.

Table 4-2 shows participant characteristics at baseline. Among the 1318 women in complete case analysis, mean age at baseline was 46.6. Women reported an average of 2.3 births. Excessive gestational weight gain in at least one pregnancy was reported by 536 (40.1%) of women. Women with a history of excessive gestational weight gain were less likely to be of Japanese or Chinese background, more likely to be smokers, and more likely to report an overweight or obese BMI prior to their first pregnancy compared to women with no excessive

gestational weight gain. Women with excessive gestational weight gain were more likely to have abdominal obesity at midlife than those with no excessive gain (49.6% versus 27.7%).

Mean follow-up time was 17.4 (standard deviation=3.8) years and the mean age of women when last observed was 64.0. Appendix B, Figures 4 and 5 show median ASCVD score and CRP by excessive gestational weight gain status across SWAN follow-up.

Analysis Models: In analysis of baseline data, a history of excessive gestational weight gain was associated with a 19.7% (95% CI= 8.5, 32.1) increase in ASCVD score (Table 4-3). This association was attenuated to 8.8% (-0.8, 19.4) with adjustment for confounders, and to 0.1% (-8.5, 9.6) in models including potential mediators. A history of excessive gestational weight gain was associated with an 85.8% (58.8, 117.4) increase in CRP in unadjusted models, which was attenuated to 41.3% (19.9, 66.5) with the addition of confounders. Mediating characteristics did not fully explain the association with CRP. Excessive gestational weight gain was associated with 20.9% (3.6, 41.0) higher CRP in models including mediators.

In longitudinal models, a history of excessive gestational weight gain was associated with a 29.6% (18.2, 42.1) increase in ASCVD score (Table 4-4). This association was attenuated to 16.1% (6.8, 26.2) with adjustment for confounders, and 9.1% (0.7, 18.2) with the further addition of potential mediators. Mean ASCVD score increased by approximately 12% per year of follow-up. There was evidence that the impact of excessive gestational weight gain on ASCVD lessened slightly over time. We observed a statistically significant interaction between the exposure and time of -0.6% change per year of follow-up consistent across models.

A history of preterm birth and history of gestational hypertension showed similar effect sizes to excessive gestational weight gain on ASCVD score with estimates of 12.3% (3.6, 21.7) and 13.1% (3.8, 23.3), respectively, adjusted for confounders. A history of gestational diabetes

was associated with a 35.7% (20.7, 52.4) increase in ASCVD score. There was no evidence of an effect of parity on ASCVD score (beta= 0.0, 95% CI= -2.6, 2.7).

Figure 4-2 shows confounder-adjusted least squares means of ASCVD score by excessive gestational weight gain status back-transformed to the original scale for interpretation. Scores are shown as percent 10-year risk. With the follow-up time set to 0 years (corresponding to SWAN baseline), the mean ASCVD score for those with no excessive gestational weight gain was 1.0% (0.9, 1.1) compared to 1.1% (1.1, 1.2) for those with a history of excessive gain in confounder-adjusted models. When follow-up time was set to 20 years, the mean confounder-adjusted risk rose to 9.5% (8.9, 10.1) and 9.8% (9.2, 10.5), respectively.

Excessive gestational weight gain was associated with an 89.2% (63.2, 119.4) increase in CRP in longitudinal models (Table 4-5). This was attenuated to 49.9% (29.9, 73.1) with the addition of confounders, and to 31.5% (15.0, 50.3) with the addition of potential mediators. There was little evidence that mean CRP changed substantively over time (beta= 0.6%, CI= 0.0, 1.2% change per year of follow-up). The impact of excessive gestational weight gain decreased over time, at an estimated rate of -1.2% (-1.9, -0.5) per year follow-up, adjusted for confounders.

Other reproductive health characteristics showed weak relationships with CRP. Parity, preterm birth, and gestational diabetes were each associated with less than 10% change in CRP. Gestational hypertension was associated with an 19.6% (-1.1, 44.5) increase in CRP, adjusted for confounders.

Figure 4-3 presents confounder-adjusted least squares mean CRP by excessive gestational weight gain status back-transformed to the original scale (mg/L). The mean CRP level for those with no excessive gestational weight gain at 0 years follow-up was estimated at 1.64 mg/L (1.44, 1.88), compared to 2.46 mg/L (2.13, 2.85) among those with excessive gestational weight gain

(Figure 4-3). At 20-years follow-up, the confounder-adjusted mean was 1.85 (1.61, 2.14) and 2.20 (1.89, 2.57) for never- and ever-had excessive gestational weight gain, respectively.

Sensitivity Analysis: Table 4-6 shows pooled estimates from confounder-adjusted longitudinal models using imputed data. In imputed data models, a history of excessive gestational weight gain was associated with a 12.7% (2.2, 24.4) higher ASCVD score and a 33.9% (18.3, 51.7) higher CRP value, adjusted for confounders.

4.5 Discussion

In this multi-ethnic cohort of parous women, a history of excessive gestational weight gain was associated with a small but statistically significant increase in ASCVD score, and a moderate, statistically significant increase in mean CRP across midlife. Women who reported one or more pregnancies with excessive gestational weight gain were estimated to have an absolute increase in 10-year ASCVD risk of 0.3 %-points compared to those without (9.8% versus 9.5% mean risk), and a 0.35 mg/l higher mean CRP (2.20 versus 1.85 mg/l), at 20 years of midlife follow-up independent of demographic, behavioral, and reproductive confounders.

Despite a large and consistent literature linking excessive gestational weight gain to later maternal obesity, there remains a lack of compelling evidence that this translates into substantive change in other traditional cardiovascular risk factors. The small impact of excessive gestational weight gain on ASCVD score that we observed may reflect differing effects of gestational weight gain on specific score components. Further research is necessary to better understand the influence of pregnancy characteristics on atherosclerotic development.

Our results are interesting in the context of recent research in the ARIC cohort, which found that having favorable inflammation and lipid profiles were commonly discordant.[215] Approximately half of those with a favorable lipid profile in the ARIC analysis had elevated CRP. The ARIC analysis also emphasized the importance of inflammation as a contributor to CVD. High CRP independently predicted atherosclerotic CVD in those with and without a favorable lipid profile over a similar follow-up period to our study.[215]

As expected, ASCVD scores increased with age and time over follow-up in this analysis. However, there was little evidence of an increase in mean CRP with age or aging. Mean CRP increased by less than 1% per year in longitudinal models and there was no effect of age at baseline. Prior studies have found chronic inflammation to increase with age in older populations.[216,217] The SWAN midlife cohort may have been too young to observe this effect.

Hypertensive disorder in pregnancy and gestational diabetes contributed to ASCVD risk and inflammation in this analysis. This is consistent with well-established links between hypertensive disorders of pregnancy and CVD risk,[54,62,67,218,219] and complications with type-2 diabetes risk.[62,220,221] Our analysis adds to this literature evidence that excessive gestational weight gain contributes to maternal cardiovascular risk at a similar magnitude as pregnancy complications. Further, our estimates suggest that excessive gestational weight gain does not primarily operate via a pathway including those complications.

Notably, we did not observe a relationship between parity and ASCVD risk or inflammation in this cohort. Among parous women, higher number of births has often been linked to CVD risk.[59] Studies on paternal CVD risk suggests the association may be due to residual confounding with socioeconomic status or factors related to child rearing as opposed to child bearing.[62,222] In addition, most studies do not account for gestational weight gain adequacy when

evaluating the role of parity.[59,223-225] Our results suggest that parity may be acting as a proxy for other reproductive health characteristics as opposed to representing a risk factor itself.

We hypothesized that the effect of excessive gestational weight gain on long-term maternal cardiovascular risk would be mediated through abdominal obesity in midlife. Our estimates partially supported this hypothesis but suggest that some association remains after controlling for midlife obesity. The mechanistic pathways currently supported in the literature connecting excessive gestational weight gain and long-term maternal cardiovascular health are through a high-risk phenotype of obesity[114,115,119] or pregnancy complications.[226,227] We reason, therefore, that the remaining association is due to residual confounding with sociodemographic health disparities or by our reliance on self-report of pregnancy complications.

Our study has many strengths. Our analysis represented a mean prospective follow-up of 17 years across midlife. This allowed us to estimate the level and change over time of the cardiovascular risk outcomes, and whether the impact of excessive gestational weight gain on each outcome changed over time. The SWAN data were also an asset in that they provide a rich source of information on participants. This allowed us to account for a wide variety of characteristics that are likely strong confounders but often difficult to measure such as physical activity and diet. Finally, we had high-quality outcome measures collected in a clinical setting by trained staff.

The following limitations should be considered in interpreting these results. Our primary analytic sample excluded over 40% of eligible participants due to missing data. However, we were able to control for many characteristics related to participant attrition such as socioeconomic status, health behaviors, and stress. Sensitivity analyses that included multiply imputed data attenuated estimates but did not impact interpretation compared to results from the complete case sample. A second limitation to this research is the use of self-recalled measures of reproductive history.

Validation research has shown that self-recall of pregnancy weight characteristics often leads to over-estimating the prevalence of excessive gestational weight gain.[128] This trend has generally not been found to bias associations between gestational weight gain adequacy and birth outcomes.[128] Because participants were unlikely to be aware of their ASCVD risk or inflammation level when reporting pregnancy weight, the concern that misreporting would vary by outcome status is limited.

4.6 Conclusion

In summary, our study found that a history of excessive gestational weight gain was associated with a small increase in maternal ASCVD risk score and moderate increase in CRP over 20 years of prospective midlife follow-up, independent of demographic characteristics and health behaviors. This adds evidence to the importance of clinical focus on healthy weight gain during pregnancy to promote long-term maternal health. Further research is necessary to understand the mechanistic pathway between pregnancy weight characteristics and maternal cardiovascular risk.

4.7 Tables and Figures

Table 4-1 Institute of Medicine Recommendations for Total Weight Gain during Pregnancy

Pre-pregnancy BMI Category	BMI Category Definition (kg/m2)		Range for Adequate Total GWG (lbs)[1]	Range for Adequate GWG Rate, 2nd and 3rd Trimester (lbs/week)[1, 2]
	NH White, NH Black, and Hispanic	*Japanese and Chinese ethnicity*		
Underweight	< 18.5	< 18.5	28–40	1.0-1.3
Normal weight	18.5-24.9	18.5-22.9	25–35	0.8-1.0
Overweight	25.0-29.9	23.0-24.9	15–25	0.5-0.7
Obese (all classes)	≥ 30.0	≥ 25.0	11–20	0.4-0.6

Abbreviations: BMI, body mass index; GWG, gestational weight gain; lbs, pounds; NH: Non-Hispanic.
1. Adequacy ranges: Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines; Rasmussen KM, Yaktine AL, editors. *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington (DC): National Academies Press, 2009.
2. Calculation assumes 4.4 lbs weight gained in first trimester.

Table 4-2 Aim 3 Participant Characteristics at SWAN Baseline (Complete Cases)

	Total	Pregnancies with Excessive GWG	
	(n=1318)	None (n=782)	One or More (n=536)
Age, mean (SD)	46.6 (2.64)	46.8 (2.67)	46.2 (2.56)
Race/Ethnicity, n (%)			
Black	358 (27.2)	194 (24.8)	164 (30.6)
White	615 (46.7)	347 (44.4)	268 (50.0)
Chinese	123 (9.3)	88 (11.3)	35 (6.5)
Hispanic	90 (6.8)	54 (6.9)	36 (6.7)
Japanese	132 (10.0)	99 (12.7)	33 (6.2)
Education, n (%)			
High school or less	298 (22.6)	170 (21.7)	128 (23.9)
Some college or degree	727 (55.2)	435 (55.6)	292 (54.5)
Post-college study	293 (22.2)	177 (22.6)	116 (21.6)
Smoking Status, n (%)			
Never smoker	817 (62.0)	521 (66.6)	296 (55.2)
Past smoker	324 (24.6)	166 (21.2)	158 (29.5)
Current smoker	177 (13.4)	95 (12.1)	82 (15.3)
Difficulty Paying for Basics, n (%)			
Not very hard	828 (62.8)	498 (63.7)	330 (61.6)
Somewhat or very hard	490 (37.2)	284 (36.3)	206 (38.4)
Menopausal Status, n (%)			
Pre-menopause	732 (55.5)	427 (54.6)	305 (56.9)
Early perimenopause	586 (44.5)	355 (45.4)	231 (43.1)
Perceived Stress Score, mean (SD)	8.5 (2.92)	8.4 (2.86)	8.5 (3.01)
Total Caloric Intake (kcal), mean (SD)	1834.9 (743.1)	1830.4 (756.2)	1841.5 (724.2)
Physical Activity Score, mean (SD)	7.8 (1.76)	7.9 (1.78)	7.7 (1.72)
Parity, mean (SD)	2.3 (1.07)	2.3 (1.09)	2.4 (1.04)
Age Pregnant First Time, mean (SD)	24.0 (5.46)	24.3 (5.42)	23.5 (5.48)
Years between last birth and baseline, mean (SD)	15.0 (6.76)	15.3 (6.89)	14.5 (6.54)
BMI Category prior to first pregnancy, n (%)			
Underweight	164 (12.4)	109 (13.9)	55 (10.3)
Normal	971 (73.7)	611 (78.1)	360 (67.2)
Overweight	127 (9.6)	43 (5.5)	84 (15.7)
Obese	56 (4.2)	19 (2.4)	37 (6.9)
Number of Excessive GWG Pregnancies, n (%)			
0	782 (59.3)	782 (100.0)	0 (0.0)
1	307 (23.3)	0 (0.0)	307 (57.3)
2	155 (11.8)	0 (0.0)	155 (28.9)
3 +	74 (5.6)	0 (0.0)	74 (13.8)
History of preterm birth, n (%)	149 (11.3)	77 (9.8)	72 (13.4)
History of inadequate GWG, n (%)	547 (41.5)	464 (59.3)	83 (15.5)

Table 4-2 Continued

History of hypertensive pregnancy, n (%)	122 (9.3)	46 (5.9)	76 (14.2)
History of gestational diabetes, n (%)	63 (4.8)	27 (3.5)	36 (6.7)
Abdominal obesity at midlife, n (%)	483 (36.6)	217 (27.7)	266 (49.6)
ASCVD score: % 10-year risk, median (25th, 75th pctl)	0.08 (0.05, 0.15)	0.08 (0.05, 0.14)	0.10 (0.05, 0.18)
CRP mg/L, median (25th, 75th pctl)	1.5 (0.53, 4.99)	1.2 (0.53, 3.52)	2.6 (0.76, 7.66)

Table 4-3 Estimated Percent Change in ASCVD Score and CRP for Ever- versus Never-Had Excessive Gestational Weight Gain (n=1318)

ASCVD Score	Estimate (95% CI)
Model 1[1]	19.7 (8.5, 32.1)
Model 2	8.8 (-0.8, 19.4)
Model 3	5.9 (-3.5, 16.1)
Model 4	0.1 (-8.5, 9.6)

CRP	Estimate (95% CI)
Model 1[1]	85.8 (58.8, 117.4)
Model 2	41.3 (19.9, 66.5)
Model 3	40.1 (18.7, 65.4)
Model 4	20.9 (3.6, 41.0)

1. Covariate structure:
Model 1 is unadjusted. Model 2 adjusted for: race/ethnicity, site, age, difficulty paying for basics, education level, caloric intake, physical activity, stress score, smoking history, BMI prior to first pregnancy, age first pregnant, years between last birth and baseline visit, parity, history of inadequate gestational weight gain, and menopause status. CRP model also adjusts for current statin use. Model 3 adjusted for: model 2 covariates as well as history of hypertensive disorder in pregnancy, history of gestational diabetes, and history of preterm birth. Model 4 adjusts for model 3 covariates and baseline abdominal obesity.

Table 4-4 Linear Mixed Model Estimates of Percent Change in ASCVD Score per Unit Increase in Exposure (n=1318)

Model 1	Estimate (95% CI)
Excessive GWG	29.6 (18.2, 42.1)
Baseline age	11.9 (10.5, 13.3)
Years of follow-up	12.2 (11.9, 12.5)
GWG*Years follow-up	-0.6 (-1.0, -0.1)
Model 2	
Excessive GWG	16.1 (6.8, 26.2)
Baseline age	12.1 (10.8, 13.4)
Years of follow-up	12.0 (11.6, 12.3)
GWG*Years follow-up	-0.6 (-1.0, -0.2)
Parity (per birth)	0.2 (-2.5, 3.0)
Model 3	
Excessive GWG	13.5 (4.5, 23.3)
Baseline age	12.4 (11.1, 13.7)
Years of follow-up	12.0 (11.6, 12.3)
GWG*Years follow-up	-0.6 (-1.0, -0.2)
Parity (per birth)	0.0 (-2.6, 2.7)
Preterm birth history	12.3 (3.6, 21.7)
Gest. hypertension	13.1 (3.8, 23.3)
Gest. diabetes	35.7 (20.7, 52.4)
Model 4	
Excessive GWG	9.1 (0.7, 18.2)
Baseline age	12.0 (10.8, 13.2)
Years of follow-up	12.0 (11.6, 12.3)
GWG*Years follow-up	-0.6 (-1.0, -0.1)
Parity (per birth)	-0.7 (-3.3, 1.9)
Preterm birth history	12.1 (3.7, 21.1)
Gest. hypertension	11.4 (2.5, 21.1)
Gest. diabetes	33.6 (19.4, 49.5)
Baseline obesity	32.2 (25.0, 39.8)

Model 1 is adjusted for variables shown. Models 2 - 4 are adjusted for variables shown as well as race/ethnicity, site, education, baseline caloric intake, history of smoking at baseline, BMI category prior to the first pregnancy, age first pregnant, years between last birth and baseline, history of inadequate gestational weight gain, and the following time-varying characteristics: physical activity score, stress score, difficulty paying for basics, menopause status, and hormone use.

Table 4-5 Linear Mixed Model Estimates of Percent Change in CRP per Unit Increase in Exposure (n=1318)

Model 1	Estimate (95% CI)
Excessive GWG	89.2 (63.2, 119.4)
Baseline age	1.4 (-0.9, 3.9)
Years of follow-up	0.4 (-0.1, 0.8)
GWG*Years follow-up	-1.2 (-1.9, -0.5)
Model 2	
Excessive GWG	49.9 (29.9, 73.1)
Baseline age	2.1 (-0.5, 4.6)
Years of follow-up	0.6 (0.0, 1.2)
GWG*Years follow-up	-1.2 (-1.9, -0.5)
Parity (per birth)	1.2 (-4.6, 7.3)
Model 3	
Excessive GWG	49.0 (28.9, 72.1)
Baseline age	2.2 (-0.3, 4.8)
Years of follow-up	0.6 (0.0, 1.2)
GWG*Years follow-up	-1.2 (-1.9, -0.5)
Parity (per birth)	1.0 (-4.8, 7.2)
Preterm birth history	-7.4 (-22.3, 10.3)
Gest. hypertension	19.6 (-1.1, 44.5)
Gest. diabetes	9.2 (-15.5, 41.2)
Model 4	
Excessive GWG	31.5 (15.0, 50.3)
Baseline age	1.3 (-1.1, 3.7)
Years of follow-up	0.7 (0.1, 1.2)
GWG*Years follow-up	-1.1 (-1.8, -0.4)
Parity (per birth)	-1.5 (-6.8, 4.1)
Preterm birth history	-6.5 (-20.6, 10.1)
Gest. hypertension	12.9 (-5.4, 34.8)
Gest. diabetes	6.3 (-16.4, 35.1)
Baseline obesity	138.9 (112.3, 168.8)

Model 1 is adjusted for variables shown. Models 2 - 4 are adjusted for variables shown as well as race/ethnicity, site, education, history of smoking at baseline, baseline caloric intake, BMI category prior to the first pregnancy, age first pregnant, years between last birth and baseline, history of inadequate gestational weight gain, and the following time-varying characteristics: statin use, physical activity score, stress score, difficulty paying for basics, menopause status, and hormone use.

Table 4-6 Longitudinal Pooled Estimates of Percent Change of ASCVD score and CRP per Unit Increase in Exposure, Confounder-Adjusted (n=2283)

		Imputed Estimate Range	
ASCVD Score	Pooled Estimate (95% CI)	Minimum	Maximum
Excessive GWG	12.7 (2.2, 24.4)	6.3	18.5
Baseline age	11.3 (10.2, 12.5)	11.3	11.4
Years of follow-up	11.4 (11.1, 11.7)	11.3	11.5
GWG*Years follow-up	-0.5 (-1, 0.0)	-0.7	-0.2
		Imputed Estimate Range	
CRP	Pooled Estimate (95% CI)	Minimum	Maximum
Excessive GWG	33.9 (18.3, 51.7)	30.0	39.4
Baseline age	0.5 (-1.5, 2.5)	0.2	0.7
Years of follow-up	0.2 (-0.3, 0.7)	0.1	0.3
GWG*Years follow-up	-0.8 (-1.4, -0.1)	-1.0	-0.5

Models are adjusted for variables shown as well as race/ethnicity, site, education, history of smoking at baseline, baseline caloric intake, BMI category prior to the first pregnancy, age first pregnant, years between last birth and baseline, parity, history of inadequate gestational weight gain, and the following time-varying characteristics: physical activity score, stress score, difficulty paying for basics, menopause status, and hormone use. CRP model is also adjusted for time-varying statin use.

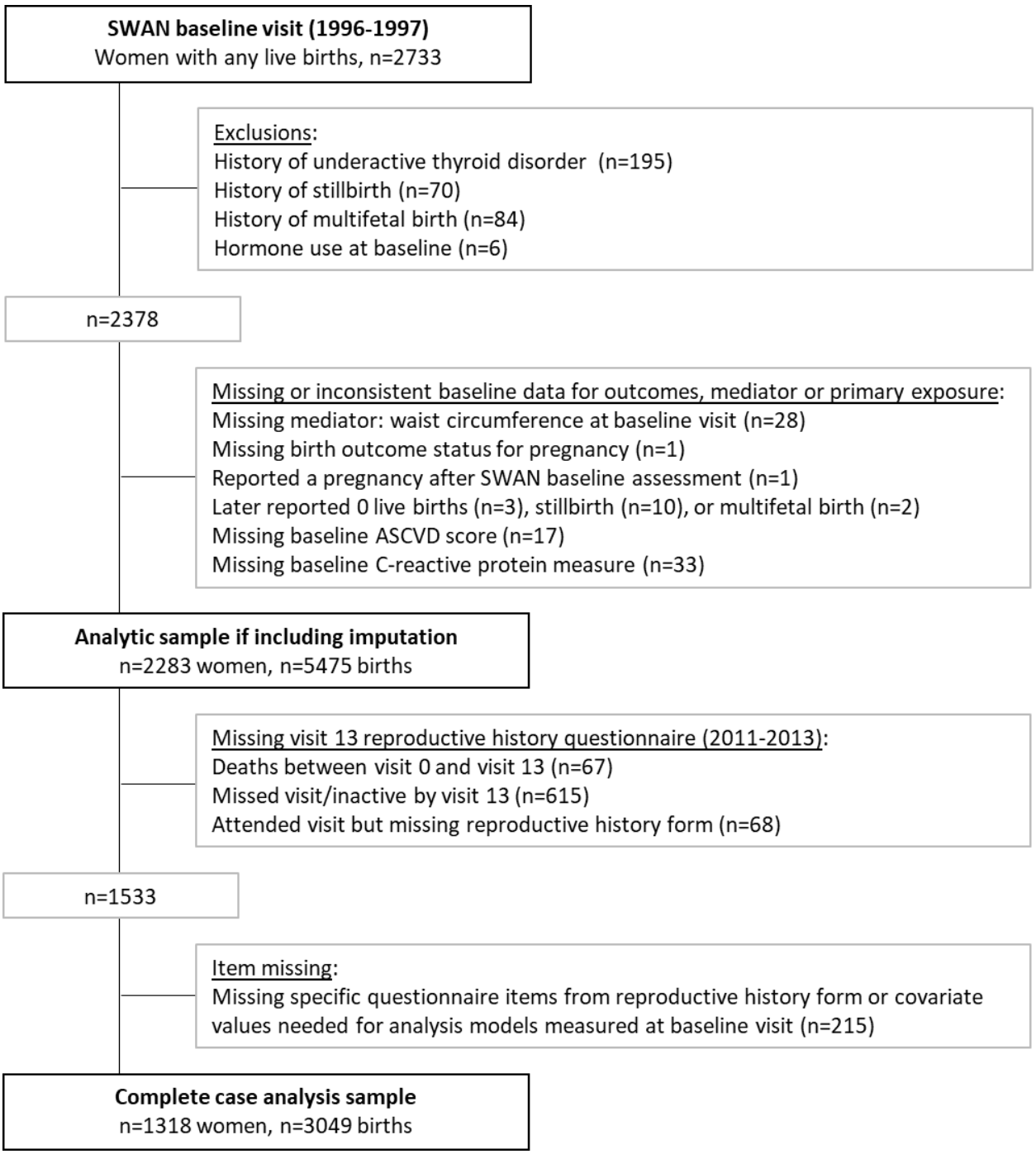

Figure 4-1 Aim 3 Participant Flow Chart

Figure details eligibility and exclusions for participants included in the complete case sample (primary analysis) and sample used in pooled imputation results (sensitivity analysis).

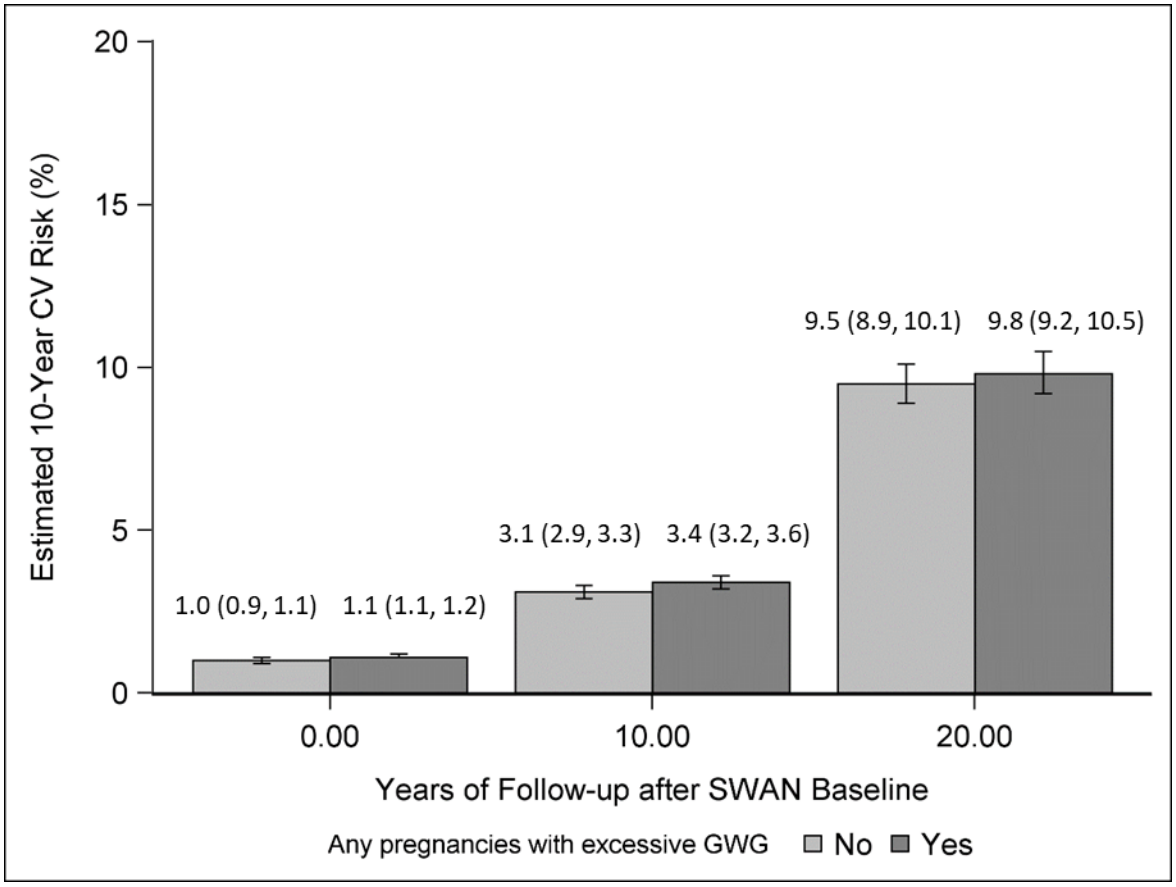

Figure 4-2 Confounder-adjusted Least Squares Mean ASCVD risk (%), n=1318

Figure shows least squares means with 95% confidence intervals of percent 10-year atherosclerotic CVD risk for those without (light grey bars) and with (dark grey bars) a history of excessive gestational weight gain. Each set of least square means is estimated on the log scale in separate linear mixed models with follow-up time set to years of SWAN follow-up shown in the x-axis. Log-scale means are back-transformed to the original scale and presented as %.

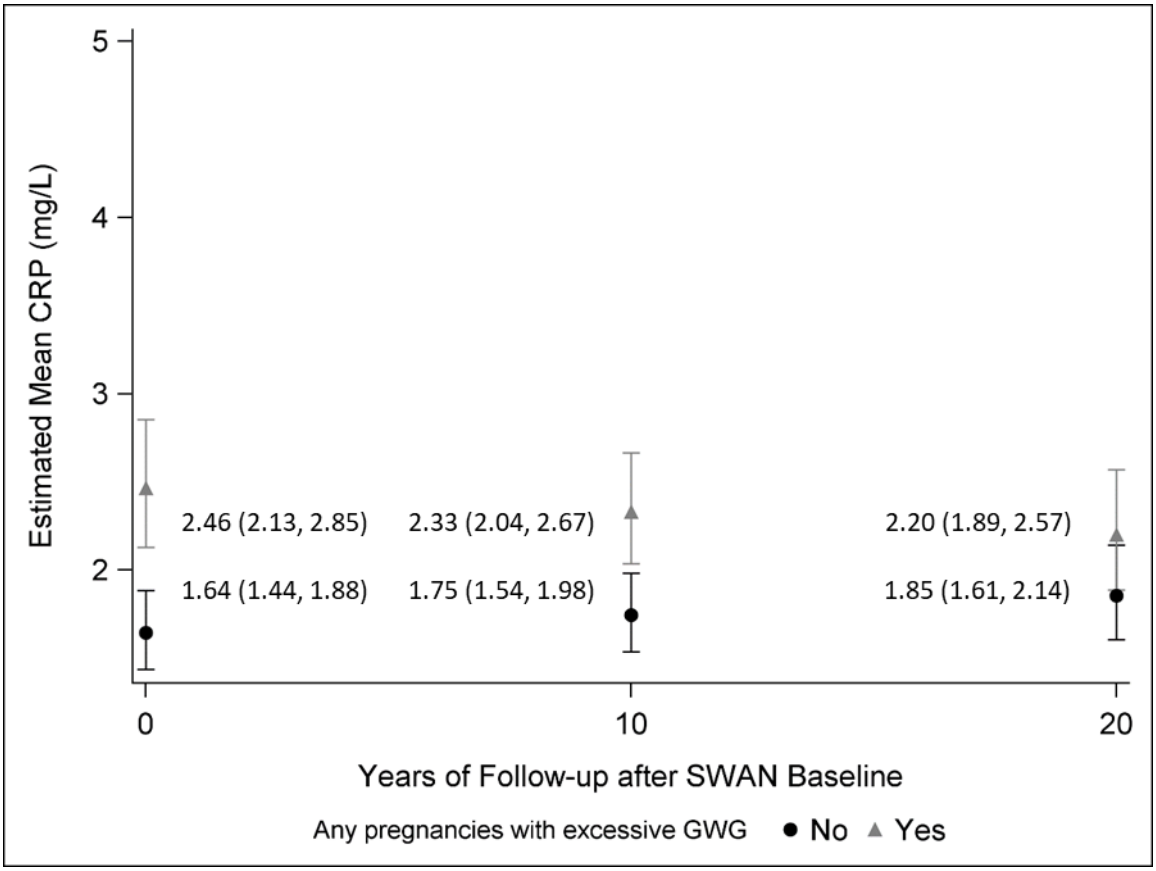

Figure 4-3 Confounder-adjusted Least Squares Mean CRP (mg/L), n=1318

Figure shows least squares means with 95% confidence intervals of CRP level for those without (black circle) and with (grey triangle) a history of excessive gestational weight gain. Each set of least square means is estimated on the log scale in separate linear mixed models with follow-up time set to years of SWAN follow-up shown in the x-axis. Log-scale means are back-transformed to the original scale (mg/L).

5.0 Discussion

5.1 Major Findings

Our goal in this dissertation is to evaluate whether excessive gestational weight gain (GWG) increases maternal susceptibility to a high-risk phenotype of obesity. We aimed to identify key associations on the potential pathway between excessive GWG, maternal midlife obesity, and cardiovascular disease (CVD). Further, we evaluated whether systematic error accounted for observed associations between excessive GWG and midlife obesity by quantifying statistical bias around those estimates.

First, we evaluated whether excessive GWG in multiple pregnancies has a cumulative, long-term impact on maternal BMI. In a cohort of parous participants in the Study of Women's Health Across the Nation (SWAN), we found that each pregnancy with excessive GWG was associated with a 0.02 (se=0.01) log-BMI unit increase at a mean age of 46.6, independent of demographic characteristics, health behaviors, and parity. The estimated marginal mean BMI increased monotonically from 25.4 kg/m^2 (95% CI=24.9, 25.9) for women with no excessive GWG pregnancies to of 28.8 kg/m^2 for those reporting excessive GWG in three pregnancies (95% CI=27.3, 30.5), adjusted for confounders. Notably, the association between excessive GWG pregnancies and maternal midlife BMI was not found to vary by parity or by race/ethnicity.

Our research also provides an applied example of methods to quantify statistical bias in life course research. The data used in our aim 1 paper were limited in ways that are common in secondary analysis studies of cohorts. Specifically, data were limited by missingness from loss to

follow-up and reliance on self-recall for some measures. To address missing data, we imputed values for gestational weight gain adequacy for the 42% of eligible participants with missing data. Pooled regression estimates were similar to those observed using only complete cases. In confounder-adjusted models a history of excessive GWG was associated with a relative risk of 1.40 (1.13, 1.74) for midlife obesity in complete case analysis and 1.38 (1.16, 1.64) from pooled estimates using imputed data. We then quantified bias due to error in self-recalled pregnancy weight. By sourcing sensitivity and specificity values for self-recalled pregnancy weight from the validation literature to inform weighted regressions, we found that plausible misclassification rates moved estimates of association away from the null. Only misclassification-adjusted models assuming 10-point lower sensitivity and 20-point higher specificity among those without midlife obesity compared to those with obesity moved the confidence interval for the relative risk to include a null result.

Finally, we asked if excessive GWG increased long-term CVD risk. To evaluate this question, we leveraged over twenty years of prospective SWAN follow-up across participants' midlife. We found that a history of excessive GWG was associated with a 16.1% (6.8, 26.2) increase in log-transformed atherosclerotic CVD risk score, and 49.9% (29.9, 73.1) higher mean log-transformed C-reactive protein in confounder-adjusted longitudinal models. This translated into an estimated marginal mean of 10-year atherosclerotic CVD risk of 9.8% (9.2, 10.5) versus 9.5% (8.9, 10.1) and mean C-reactive protein of 2.20 mg/l (1.89, 2.57) versus 1.85 mg/l (1.61, 2,14) at 20 years of midlife follow-up for those with and without excessive GWG, respectively. We interpreted this result to illustrate a small, but not clinically meaningful, increase in atherosclerotic CVD risk and a moderate increase in C-reactive protein. Both associations were attenuated but remained statistically significant accounting for early midlife obesity.

5.2 Public Health Significance

The burden of CVD in the United States persists despite improvements in many traditional risk factors over recent decades. The increasing prevalence of obesity contributes to CVD rates and is highest among midlife women. We investigated GWG as a potential modifiable CVD risk factor among parous women that manifests before midlife. Our findings contribute to the epidemiologic literature and have implications for clinical practice.

Excessive GWG in multiple pregnancies had a cumulative impact on midlife maternal BMI in our data. Clinically, this finding supports prenatal care as an opportunity for obesity prevention at any point in a person's birth history. Our results frame healthy pregnancy weight through a life course perspective. Intervention to support healthy weight gain in pregnancy can be meaningful throughout the reproductive period, with health impacts continuing across midlife.

Our research highlights the need for nuance when considering obesity as a measure of cardiovascular risk. We found no clinically meaningful difference in atherosclerotic CVD risk score between participants with and without a history of excessive GWG, despite differences in obesity prevalence in the same population. From a clinical perspective it is important to remember that weight characteristics are more predictive of CVD on a population level than individual level.[228] An estimated one-third of individuals with obesity do not have metabolic complications such as hypertension, high fasting blood glucose, or dyslipidemia.[18,19,229] This metabolically healthy phenotype of obesity has been associated with intermediate CVD risk compared to metabolically-healthy individuals with a normal weight BMI and metabolically-unhealthy individuals with an obese BMI.[21,24,25,230] The heterogeneous nature of obesity as a health characteristic is not well understood.[18,20,22] The biological mechanism behind divergent risk levels

in obesity is unknown, with prominent theories highlighting differences in visceral versus subcutaneous adipose tissue or characteristics of adipose tissue growth.[22,103] These theories are plausible but have not been shown to improve clinical prediction of metabolic risk.[102,105] In addition, the stigma attached to obesity heightens the need for careful consideration in interpreting related research.

Obesity stigma is endemic socially[231-233] and in clinical practice.[234-237] This stigma has been associated with worse care and health outcomes.[235,238-240] The misconception of weight as a solely behaviorally-driven health status drives this stigma and obscures upstream factors including health disparities.[241] The view that obesity is a monolithic marker of disease also contributes.[235,240] In research, a more informative approach is to design studies to test specific pathways to disease, as opposed to treating obesity as a stand-in for overall poor health in conceptual models.

We observed higher mean levels of the inflammatory marker C-reactive protein among those with a history of excessive GWG. This pathway is of interest as inflammation is a key component of the atherosclerotic process. Our results contribute the finding that chronic inflammation may continue for years after the last birth. We also observed that as women near early late life those with and without excessive GWG become more alike in their inflammatory profile. The biological process linking GWG and long-term maternal inflammation is unclear. Excessive GWG may contribute to a phenotype of obesity that favors the production of pro-inflammatory adipokines. Alternatively, excessive GWG may trigger an imbalance in cytokine production in pregnancy that lasts into the postpartum period.

We consistently observed null results when estimating the effect of parity on long-term maternal health. Among the parous women in the SWAN cohort, number of births was not associated with risk of obesity, CVD risk score, or inflammation independent of demographic,

behavioral, and other reproductive characteristics. The estimated effect of excessive GWG on early midlife BMI did not vary by parity. Previous literature on parity and maternal CVD risk is conflicting, despite decades of research on the topic.[57,59] Our results suggest that parity is not an independent risk factor but instead acts as a proxy for exposure to other reproductive characteristics. Preterm birth, hypertensive disorder in pregnancy, gestational diabetes, and excessive GWG independently contributed to maternal CVD outcomes in our models.

This research observes CVD risk during a critical period for women. In midlife, the accumulation of cardiovascular risk factors with age is augmented by the menopause transition. The menopause transition generally begins in the mid-40s and is characterized by irregularity in the menstrual cycle. This is driven by changes in reproductive hormones. In the years surrounding the final menstrual period (FMP), the level of estradiol declines while follicle-stimulating hormone increases. These trends vary in level and rate of change, with some women experiencing a temporary increase in estradiol prior to the FMP.[242] It is unclear whether endogenous hormone levels themselves influence CVD risk.[243] However, the menopause transition is a time of substantive changes in cardiovascular risk factors independent of chronological age[244]

Previous literature has observed a number of changes in CVD risk during the menopause transition. Subclinical markers of atherosclerosis including carotid intima-media thickness may increase as women near the FMP,[245] with some evidence of greater progression among women who transition from pre to post menopause more quickly.[246] Changes in risk factors during midlife such as worsening lipid profile and increased fat mass compared to lean mass accelerate in the years surrounding the FMP independent of age.[247-249]

Characteristics of the menopause transition are also associated with CVD development. Meta-analyses have indicated that early age at menopause increases risk of CVD.[250] Specific

patterns of hormone change around the FMP may contribute to atherosclerotic progression.[251] Vasomotor symptoms, such as hot flashes and night sweats, are associated with higher lipid levels[252] and insulin resistance.[253] These symptoms may be a marker of endothelial dysfunction and vascular vulnerability.[254] Finally, hormone therapy used to manage menopause symptoms may increase CVD risk, especially among older women or those further from the FMP.[255]

It is essential to consider menopause stage and hormone use when evaluating other risk factors for CVD among women in midlife. We quantified the influence of earlier reproductive characteristics on midlife cardiovascular health, accounting for chronological age, menopause status, and hormone therapy use. By incorporating these measures, we contribute well-characterized estimates of CVD risk profile across this dynamic stage of the life course.

Methodologically, our work illustrates the importance of quantitative bias analysis in epidemiologic research. Because describing the relationship between an exposure and outcome is a fundamental component of epidemiology, understanding the extent to which our estimates represent a true association is paramount. Systematic error in data collection can bias estimates away from the true association and change inference. Quantifying bias around a result improves a study's validity to inform public health policy.[176,256] The potential for quantitative bias analysis to strengthen regulatory[257] and peer-review processes has also been described.[258] Despite the accessibility of methods to analyze bias, epidemiologic manuscripts rarely incorporate them, relying instead on qualitative descriptions of the potential for systematic error.[144,259,260]

We quantified potential bias from two sources of systematic error common in observational studies: missing data and misclassification. We provided a rare applied example in which both sources of error occurred in the primary exposure as opposed to the outcome. Our analysis presented a range of plausible values for the association between excessive GWG and maternal

midlife obesity, describing the degree to which our estimates were susceptible to bias. By incorporating quantitative bias analysis, observational data such as the SWAN cohort can inform robust epidemiologic research even in the presence of likely systematic error.

In summary, we found evidence that excessive GWG increased the risk of maternal midlife obesity. Our results were robust to common sources of systematic error. We contribute the novel finding that excessive GWG contributes to chronic inflammation among parous women up to 3 decades following the last birth. However, increased risk of obesity did not translate into a clinically meaningful difference in atherosclerotic CVD risk score in our longitudinal analysis.

5.3 Strengths and Limitations

The comprehensive nature of the SWAN study is a fundamental strength of this research. We were able to leverage data on physiologic, behavioral, and social characteristics in analytic models. The availability of important factors such as health insurance access and social support were critical to our ability to model the probability of participant attrition in imputation models [126,183,186]. This rich data further allowed us to estimate longitudinal associations with outcomes collected prospectively over two decades of participants' lives. Our work highlights the continued importance of cohort studies and their ability to collect wholistic data on participants.

Available measures for outcomes and confounding factors were rigorously collected. Waist circumference, blood pressure, lipids, and other cardiovascular measures were collected in-clinic by trained staff following a shared protocol. Biomarker values were independently calibrated to be appropriate for longitudinal analysis. We also benefitted from high-quality measures of

important confounding characteristics such as diet and physical activity. Importantly, the SWAN study was designed to characterize the menopause transition, collecting high-quality prospective measures of menopause status based on bleeding patterns and surgical history, as well as information on use of hormone replacement therapy. These attributes are critical to take into account when modeling CVD and inflammation in midlife.

The definition of our main exposure, excessive GWG, is a valid, literature-based measure that represents the health effects of pregnancy weight change.[31] The measure also allowed us to account for inadequate GWG. This is preferable to comparing low versus high gain, which conflates well-supported pregnancies with higher-risk inadequate GWG pregnancies.

The collection method for GWG adequacy was a limitation to this research. We relied on retrospective self-report of pre-pregnancy weight and GWG amount for each pregnancy, collected many years after the last birth. Self-recall can be a valid measure, but the agreement between recalled and prospectively measured pregnancy weight varies widely.[128] However, this provided an opportunity to assess biases common to life course research. We were able to evaluate reliability of the measure and quantify bias around estimates of association due to misclassification.

We were limited in our ability to account for pregnancy complications and preterm birth. These factors likely play a major role in the life course pathway from pregnancy to midlife that we were not able to describe well with our retrospective measures in SWAN. In addition, we do not account for breastfeeding in this analysis. There is some evidence that breastfeeding may reduce the risk of maternal diabetes[53,75,76] and CVD[55,77]. Anecdotally the effect is suggested to operate through increased postpartum weight loss, but there is little confirmatory evidence.[78,79] We chose in our analysis to focus on weight change during pregnancy, but the mitigating potential of interventions on post-partum weight retention warrant further consideration.[44,261]

5.4 Future Directions

Questions remain surrounding the role of reproductive history on cardiovascular and metabolic health. During reproductive years, the relationship between GWG adequacy, pregnancy complications, and preterm birth are not fully understood. Excessive GWG is associated with higher rates of gestational hypertension[226,262] and gestational diabetes.[226] These characteristics may be causally linked, due to underlying subclinical factors, or represent pre-existing risk. Similarly, it is unclear whether associations between pregnancy complications and maternal CVD risk[4,54,63-65] are causal or markers of underlying susceptibility.[62] This pathway is further confounded by glaring racial disparities in prenatal and maternal health in the US.[263-267] Future research including subclinical and upstream risk factors as well as prospective measures of complications are necessary to better disentangle the causal pathway between excessive GWG, complications, and maternal CVD risk.

Secondly, more research is necessary to evaluate a possible direct effect of excessive GWG on chronic inflammation outside of the pathway including obesity. In our analysis, a positive association between history of excessive GWG and C-reactive protein persisted independent of midlife obesity. This could represent residual confounding or a causal effect. Some direct effect is biologically plausible. Inflammatory regulation and change are part of pregnancy[61,268,269] and there is some evidence that pregnancy factors are associated with postpartum inflammation.[62] High GWG has been associated with increased C-reactive protein during pregnancy[270] and in breastmilk, independent of prepregnancy BMI.[271] Whether this represents cytokine imbalance from regulatory functions of pregnancy or a consequence of excess adiposity has not been studied.

Our results point to a need for further research on the development of obesity phenotypes. Only recently has interest been expressed in the literature to distinguish risk factors for high and low-risk phenotypes.[272,273] We found that excessive GWG may contribute to a phenotype of obesity with elevated inflammation. In addition to its role in atherosclerotic development, inflammation may drive the difference between metabolically healthy and unhealthy obesity phenotypes.[274,275] However, our results do not fully support this interpretation. We found that the impact of excessive GWG on CVD risk score—a score comprised of factors that define metabolically unhealthy obesity—was minimal.

In closing, prevention of excessive GWG is a practical target for intervention to support long-term cardiovascular health among people who give birth. Our research highlights key points on a hypothetical causal pathway between excessive GWG and CVD risk. More research is necessary to continue unraveling the dynamic relationships between reproductive history and cardiovascular health.

Appendix A SWAN Study Acknowledgments

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Steering Committee: Susan Johnson, Current Chair; Chris Gallagher, Former Chair.

We thank the study staff at each site and all the women who participated in SWAN.

Appendix B Supplementary Tables and Figures

Appendix Table 1. Institute of Medicine Recommendations for Total Weight Gain during Pregnancy

Prepregnancy BMI Category	BMI Category Definition (kg/m2)		Range for Adequate Total GWG (lbs)[1]	Range for Adequate GWG Rate, 2nd and 3rd Trimester (lbs/week)[1, 2]
	NH White, NH Black, and Hispanic[2]	*Japanese and Chinese ethnicity*[3]		
Underweight	< 18.5	< 18.5	28–40	1.0-1.3
Normal weight	18.5-24.9	18.5-22.9	25–35	0.8-1.0
Overweight	25.0-29.9	23.0-24.9	15–25	0.5-0.7
Obese (all classes)	≥ 30.0	≥ 25.0	11–20	0.4-0.6

Abbreviations: BMI, body mass index; GWG, gestational weight gain; lbs, pounds; NH: Non-Hispanic.
1. Adequacy ranges: Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines; Rasmussen KM, Yaktine AL, editors. *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington (DC): National Academies Press, 2009.
2. Calculation assumes 4.4 lbs weight gained in first trimester.
3. In creating the categorical GWG adequacy variables, alternative BMI cutoffs were used for Japanese and Chinese participants, with overweight defined as ≥23 kg/m^2 and obese as ≥25 kg/m^2. This is consistent with recommendations from the Western Pacific Region WHO (The Asia-Pacific perspective: redefining obesity and its treatment. Sydney: Health Communications Australia: World Health Organization, 2000.), and prior research in Japanese and Chinese populations living in North America (1. Razak F, Anand SS, Shannon H, et al. Defining obesity cut points in a multiethnic population. *Circulation* 2007;115(16):2111-8; and: 2. Palaniappan LP, Wong EC, Shin JJ, et al. Asian Americans have greater prevalence of metabolic syndrome despite lower body mass index. *International journal of obesity (2005)* 2011;35(3):393-400.).

Appendix Table 2. Aim 1 Participant Characteristics by Missing Data Status

	Included	Excluded due to Missing Data	p
Number of Women	1181	985	
Sociodemographic Characteristics			
Age, mean ± SD	46.6 ± 2.64	46.2 ± 2.73	0.001
Race/Ethnicity, n (%)			<.001
Black	309 (26.2)	348 (35.3)	
Caucasian	559 (47.3)	358 (36.4)	
Chinese	116 (9.8)	59 (6.0)	
Hispanic	72 (6.1)	149 (15.1)	
Japanese	125 (10.6)	71 (7.2)	
Education, n (%)			<.001
High school or less	253 (21.4)	346 (36.0)	
Some college/degree	652 (55.2)	485 (50.4)	
Post-college study	276 (23.4)	131 (13.6)	
Smoking Status, n (%)			<.001
Never smoker	724 (61.3)	525 (54.1)	
Past smoker	297 (25.1)	208 (21.4)	
Current smoker	160 (13.5)	237 (24.4)	
BMI in High School, median (IQR)	20.5 (19.0, 22.2)	20.5 (18.9, 22.5)	0.422
Difficulty Paying for Basics, n (%)			<.001
Very or somewhat hard	414 (35.1)	502 (51.8)	
Not very hard	767 (64.9)	467 (48.2)	
Perceived Stress Score, mean ± SD	8.4 ± 2.90	8.8 ± 3.05	0.002
Total Caloric Intake, mean ± SD	1826 ± 725.7	1914 ± 822.9	0.010
Physical Activity Score, mean ± SD	7.9 ± 1.76	7.5 ± 1.78	<.001
Reproductive History			
Parity, mean ± SD	2.3 ± 1.07	2.5 ± 1.3	<.001
Age Pregnant First Time, mean ± SD	24.1 ± 5.48	22.6 ± 5.46	<.001

Appendix Table 3. Cross Tabulation of Number of Excessive GWG Pregnancies by Parity (n=1181)

Number of Excessive GWG Pregnancies, n (%)				
	Parity			
	1	2	3	4 +
0	171 (63.1)	323 (62.5)	131 (54.8)	90 (58.4)
1	100 (36.9)	88 (17.0)	45 (18.8)	30 (19.5)
2	–	106 (20.5)	24 (10.0)	8 (5.2)
3 +	–	–	39 (16.3)	26 (16.9)
Total	271	517	239	154

Appendix Table 4. Mean Difference and Spearman Correlation Coefficients of GWG Amount Reported at SWAN Baseline Visit versus Follow-up 13 Visit, per Birth (n=1181 women, n=2620 births)

Birth Number	N (Women)	Mean (SD) Difference*	Spearman Correlation R^2	P
1	1154	1.80 (10.44)	0.73	<.001
2	884	2.36 (10.27)	0.63	<.001
3	381	2.67 (10.88)	0.65	<.001
4	150	4.18 (12.68)	0.57	<.001
5	39	7.51 (20.61)	0.16	0.341
6	7	9.00 (17.03)	0.41	0.357
7	4	-1.75 (8.30)	0.95	0.051
8	1	2.00 (.)	NA	NA

*Calculated as GWG amount self-reported at follow-up visit 13 subtracted from GWG amount self-reported at baseline (pounds).

Appendix Table 5. Change in Log-Transformed BMI at Midlife per Number of Pregnancies with Excessive Gestational Weight Gain, Restricted to Women with 1 to 4 Births (n=1139 women, n=2470 births)

Predictor	Slope	se	p
Model 1, Unadjusted			
Number Excessive GWG Pregnancies	0.079	0.007	<.001
*Model 2, Minimally Adjusted**			
Number Excessive GWG Pregnancies	0.025	0.011	0.019
Number Inadequate GWG Pregnancies	-0.016	0.006	0.008
Any Excessive GWG Pregnancies (Yes/No)	0.040	0.019	0.039
Parity	0.004	0.007	0.502
Model 3, Fully Adjusted†			
Number Excessive GWG Pregnancies	0.025	0.011	0.017
Number Inadequate GWG Pregnancies	-0.015	0.006	0.014
Any Excessive GWG Pregnancies (0/1)	0.036	0.019	0.062
Parity	0.004	0.007	0.571
Number Pregnancies with Hypertensive Disorder	0.049	0.014	0.001
Number Pregnancies with Gestational Diabetes	0.025	0.019	0.196

BMI, Body mass index; GWG, Gestational weight gain

*Model 2 adjusted for variables shown as well as study site, age at outcome measure, race/ethnicity, education, smoking, adolescent BMI, difficulty paying for basics, menopausal status, stress score, caloric intake, physical activity score, years since last birth, and age first pregnant.

†Model 3 adjusted for variables noted with Model 2 as well as number of pregnancies with hypertensive disorder and number pregnancies with gestational diabetes.

Appendix Table 6. Adjusted Odd Ratios and 95% CI of Having Missing Data

Characteristic	OR[1]	(95% CI)
Race/Ethnicity (reference: White)		
Black	1.28	(0.99, 1.65)
Chinese	0.71	(0.36, 1.40)
Hispanic	0.64	(0.33, 1.25)
Japanese	1.12	(0.63, 2.00)
Site (reference: Pittsburgh)		
Michigan	0.96	(0.67, 1.36)
Boston	1.36	(0.94, 1.98)
Chicago	2.04	(1.44, 2.88)
Davis	0.89	(0.53, 1.49)
UCLA	0.87	(0.53, 1.43)
New Jersey	2.34	(1.40, 3.90)
Low language acculturation	1.57	(1.00, 2.46)
High school or less education	1.55	(1.22, 1.95)
No health insurance	1.48	(1.02, 2.16)
Ever-smoker	1.44	(1.17, 1.75)
Peri-menopause (vs Pre-menopause)	1.18	(0.97, 1.43)
Parity (per birth)	1.13	(1.04, 1.24)
Age (years)	0.91	(0.87, 0.95)
Social support scale (0-16)	0.96	(0.94, 0.99)
Years since last birth	1.02	(1.01, 1.04)

Abbreviations: OR, odds ratio; CI, confidence interval

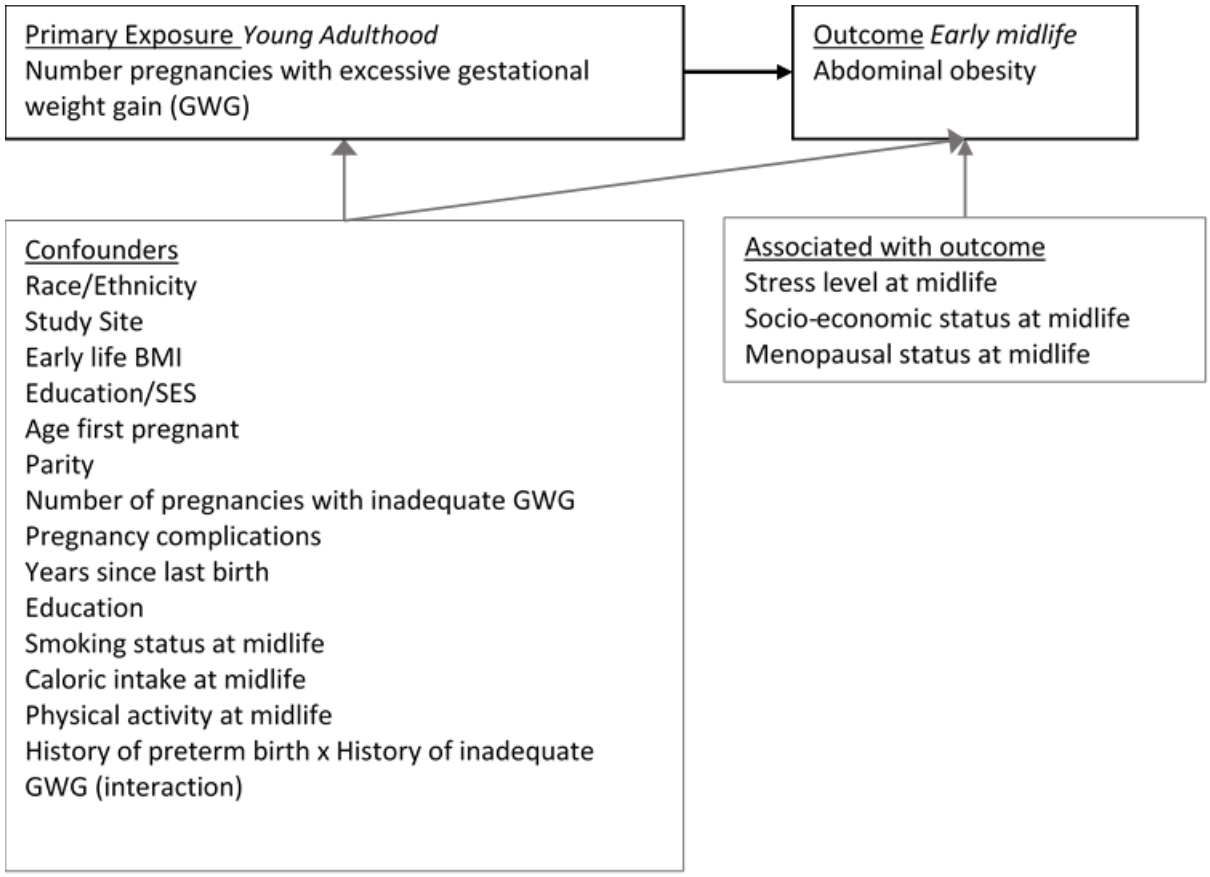
1. Model is adjusted for all characteristics shown in Table.

Appendix Table 7. Distribution of Excessive GWG and Preterm Birth, Observed versus Imputed

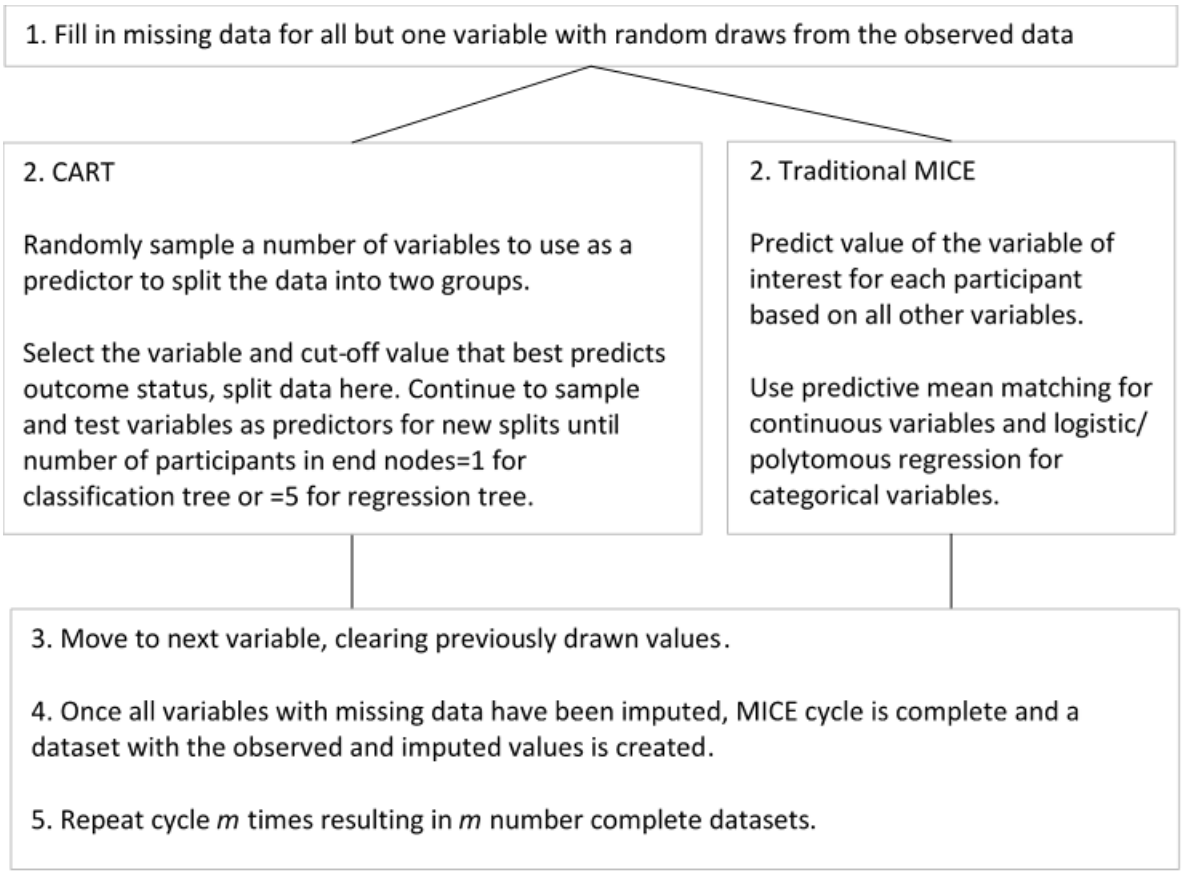
Dataset	Ever-Had Excessive GWG, n (%)	Ever-Had Preterm Birth, n (%)
Observed (n=1340)		
	544 (40.6)	151 (11.3)
Datasets Imputed with Traditional MICE (n=999)		
1	427 (42.7)	133 (13.3)
2	428 (42.8)	158 (15.8)
3	423 (42.3)	146 (14.6)
4	432 (43.2)	157 (15.7)
5	415 (41.5)	155 (15.5)
6	429 (42.9)	151 (15.1)
7	424 (42.4)	164 (16.4)
8	434 (43.4)	151 (15.1)
9	406 (40.6)	145 (14.5)
10	391 (39.1)	151 (15.1)
Datasets Imputed with CART-based MICE (n=999)		
1	420 (42.0)	156 (15.6)
2	426 (42.6)	162 (16.2)
3	442 (44.2)	147 (14.7)
4	427 (42.7)	146 (14.6)
5	397 (39.7)	134 (13.4)
6	433 (43.3)	165 (16.5)
7	421 (42.1)	156 (15.6)
8	426 (42.6)	155 (15.5)
9	429 (42.9)	157 (15.7)
10	419 (41.9)	155 (15.5)

Appendix Table 8. Aim 3 Participant characteristics at SWAN baseline stratified by missing data status

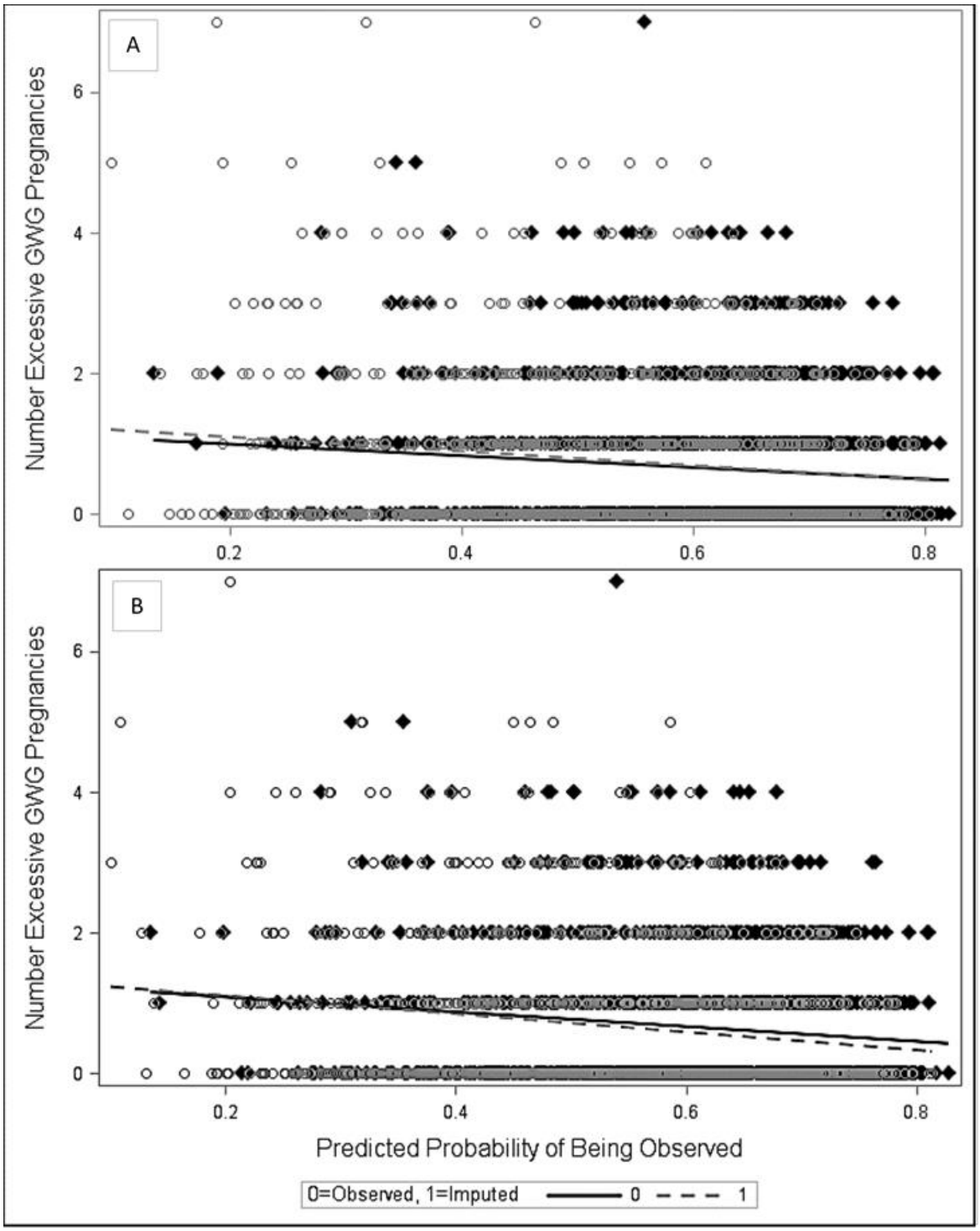
	Missing Data Status	
	Complete Cases (n=1318)	Missing Reproductive Data or Covariate(s) (n=965)
Age, mean (SD)	46.6 (2.64)	46.2 (2.70)
Race/Ethnicity, n (%)		
Black	358 (27.2)	330 (34.2)
White	615 (46.7)	360 (37.3)
Chinese	123 (9.3)	63 (6.5)
Hispanic	90 (6.8)	143 (14.8)
Japanese	132 (10.0)	69 (7.2)
Education, n (%)		
High school or less	298 (22.6)	332 (35.3)
Some college or degree	727 (55.2)	477 (50.7)
Post-college study	293 (22.2)	132 (14.0)
Smoking Status, n (%)		
Never smoker	817 (62.0)	513 (53.2)
Past smoker	324 (24.6)	207 (21.5)
Current smoker	177 (13.4)	245 (25.4)
Difficulty Paying for Basics, n (%)		
Not very hard	828 (62.8)	471 (49.5)
Somewhat or very hard	490 (37.2)	481 (50.5)
Menopausal Status, n (%)		
Pre-menopause	828 (62.8)	471 (49.5)
Early perimenopause	490 (37.2)	481 (50.5)
Perceived Stress Score, mean (SD)	8.5 (2.92)	8.8 (3.04)
Total Caloric Intake (kcal), mean (SD)	1834.9 (743.1)	1921.1 (826.4)
Physical Activity Score, mean (SD)	7.8 (1.76)	7.5 (1.78)
Parity, mean (SD)	2.3 (1.07)	2.5 (1.20)
Age Pregnant First Time, mean (SD)	24.0 (5.46)	22.6 (5.41)
Years between last birth and baseline, mean (SD)	15.0 (6.76)	15.2 (6.67)
Abdominal obesity at midlife, n (%)	483 (36.6)	438 (45.4)
ASCVD score: % 10-year risk, median (25th, 75th pctl)	0.08 (0.05, 0.15)	0.11 (0.06, 0.25)
CRP mg/L, median (25th, 75th pctl)	1.48 (0.53, 4.99)	2.36 (0.87, 7.31)



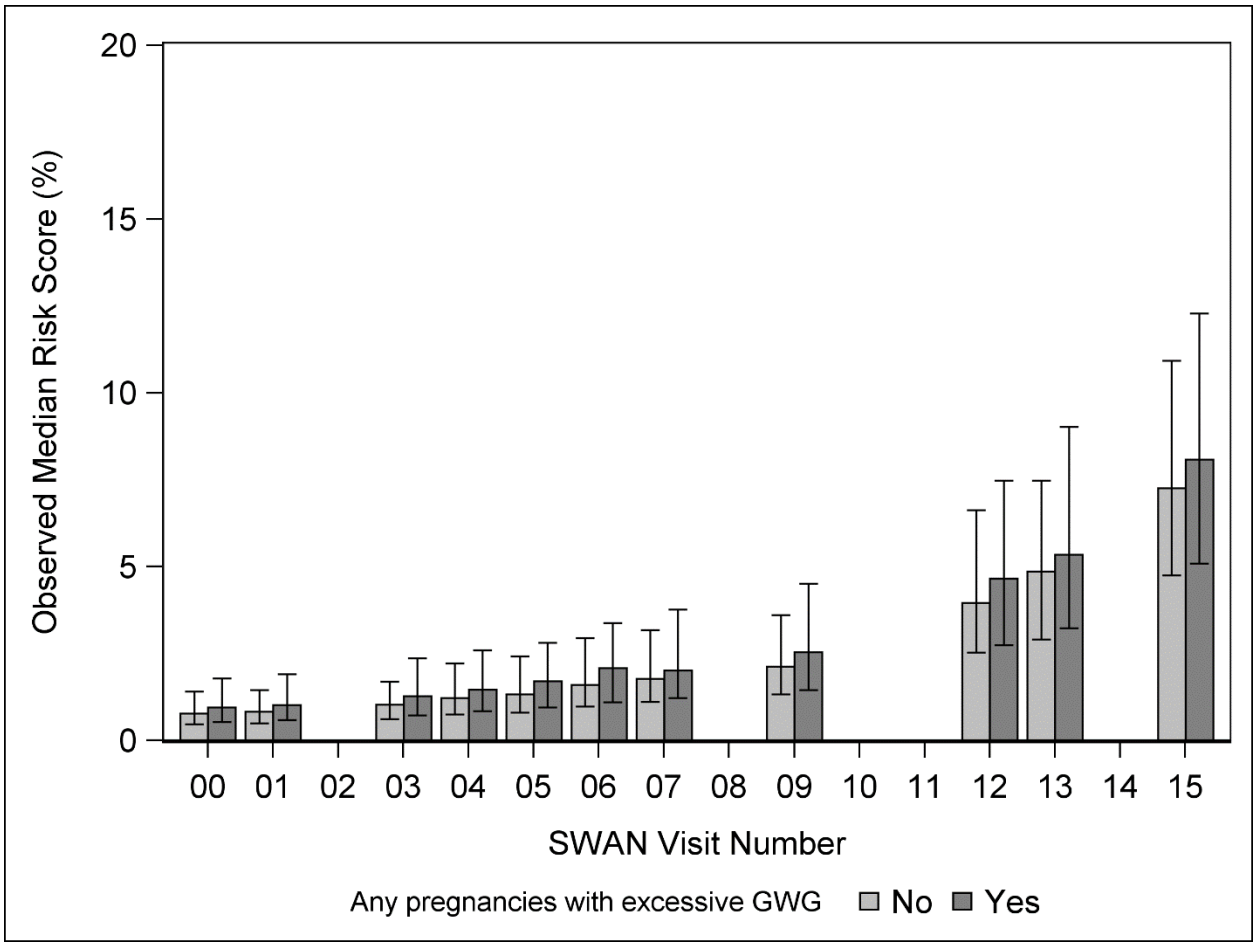
Appendix Figure 1. Directed acyclic graph of effects hypothesized in this analysis.



Appendix Figure 2. Imputation Flow Chart

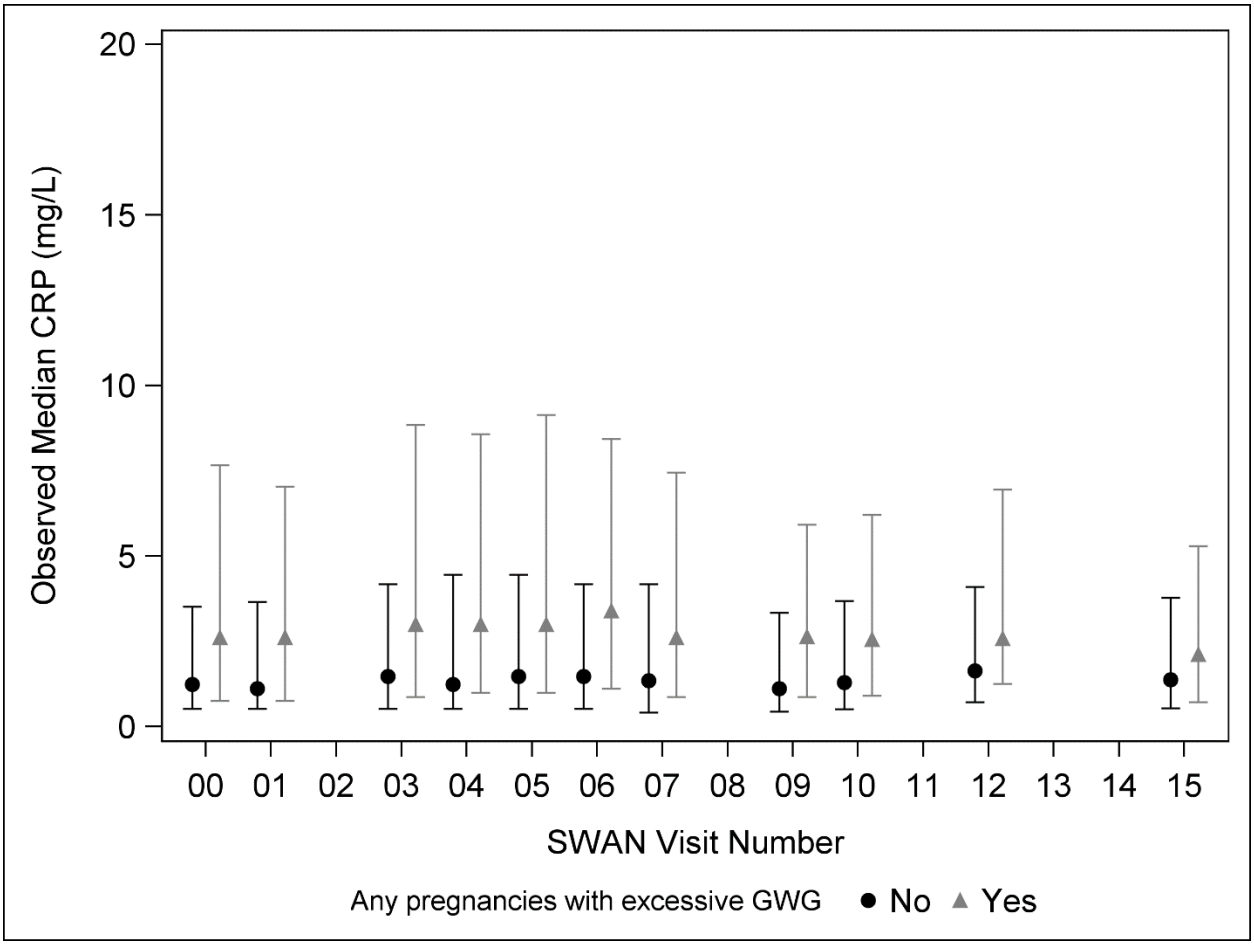


Appendix Figure 3. Scatter plot of predicted probability of being observed against number of excessive gestational weight gain pregnancies.



Appendix Figure 4. Median ASCVD score (%) with 25th and 75th percentiles, n=1318.

Figure shows values by SWAN visit, stratified by excessive gestational weight gain history.
Note that some components of ASCVD score were not measured at SWAN visits 2, 8, 10, and 11.



Appendix Figure 5. Median C-reactive protein (mg/l) with error bars to the 25th and 75th percentiles, n=1318.

Figure shows observed values by SWAN visit, stratified by excessive gestational weight gain history. Note that C-reactive protein was not measured at SWAN visits 2, 8, 11, 13, and 14.

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