Physical Activity, Sedentary Behavior, and Maternal Cardiovascular Health During Pregnancy: Impact on Placental Vascular Development

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Though adverse pregnancy outcomes are related to maternal health and behaviors during pregnancy and are associated with pathological changes in the placenta, the mechanistic origins of these relationships are unclear. An important limitation of previous research is the use of dichotomous outcomes of placental pathology that limit statistical approaches to analysis. This study aimed to describe the associations between physical activity (PA), sedentary behavior (SB), and maternal cardiovascular health (CVH) with a novel continuous measure of placental health. **Methods:** Participants (N=64) enrolled in cohort studies that prospectively measured PA and SB for one week during each trimester of pregnancy and co-enrolled in the Magee Obstetric Maternal & Infant Biobank to provide placenta tissue samples at delivery. CVH was assessed and quantified using the Life's Essential 8 scoring framework set forth by the American Heart Association. Diet, sleep, and smoking were collected in each trimester via questionnaire. Blood pressure, gestational weight gain, one-hour glucose screen, and pre-pregnancy BMI were abstracted from the medical record. At delivery, placental villous tissue was collected, formalin-fixed, and paraffin-embedded. Tissue sections were stained with CD34 antibody. Whole-slide images were scanned and analyzed using Aperio ImageScope Positive Pixel Count software. Fetal vascular percentage (FV%) was quantified as the proportion of pixels positive for CD34 antigen. Associations between exposures and FV% were examined using linear regression, with covariate adjustment for maternal age and smoking as appropriate. **Results:** No significant associations were found between PA or SB and

FV%, though associations approached significance for adjusted models of SB in the first trimester (β=0.73, p=0.128). Similarly, no significant associations between CVH scores and FV% were found either across gestation or within trimesters, though positive associations approached significance in several models (p<0.2). Higher diet score in the first trimester was significantly associated with greater FV% (β=1.0, p=0.018). No other component scores or continuous values of components were associated with FV%. **Conclusions:** Development of FV% in the placenta was not statistically associated with measures of PA, SB, or CVH. Associations approaching significance suggest repetition in a fully-powered study and that the novel FV% outcome may be a useful tool for further research.

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I dedicate this project to the participants who selflessly shared this personal time in their lives, giving us a glimpse inside their journey into motherhood.

Finally, I dedicate this dissertation to my children. Fiona and Risa, you are my guiding lights. I hope you always reach for your own stars, work hard, and make funny faces!

1.0 Introduction

1.1 Background

Cardiovascular disease is the leading cause of death among women in the United States.[1] While the causes of cardiovascular disease are multifaceted, experiencing an adverse pregnancy outcome (APO) such as hypertensive disorders of pregnancy, gestational hypertension, preterm birth, or intrauterine growth restriction increases the risk of future cardiovascular disease in the mother.[2–4] As such, pregnancy is an important time in the life course of the pregnant person; its outcome can offer clues to future maternal disease risk and can identify those for whom early screening and intervention may be most appropriate. However, the direction of the relationship between APOs and maternal cardiovascular disease is not clear. In some cases, an APO appears to be an "unmasking" of preexisting subclinical cardiovascular disease[5,6], but some evidence also suggests that pathological development of the placenta leads to negative systemic maternal cardiovascular adaptations that could contribute to long-term maternal cardiovascular risk.[7,8]

Modifiable lifestyle factors, such as physical activity, diet, and smoking, are closely related to cardiovascular health.[9,10] These factors are also related to the occurrence of APOs[11,12], and some evidence points to a relationship between modifiable cardiovascular risk metrics during pregnancy and placental vascular development.[13,14] Similar pathways of angiogenesis and vascular function as those seen in systemic circulation due to exercise may be responsible for positive vascular adaptations in the placenta.[15] However, limited data support this hypothesis. Thus, further study is needed to elucidate the mechanisms by which cardiovascular health metrics, including physical activity and sedentary behavior, impact vasculature in the placenta.

1.2 Significance and Conceptual Framework

APOs, including hypertensive disorders of pregnancy, preterm birth, and intrauterine growth restriction, are related to poor cardiovascular sequelae for both the birthing individual and the child in later life.[16] Current research points to maladaptation in the placenta as an important contributor to these disorders.[17] However, evidence-based preventive measures for APOs are limited, largely due to the fact that the precise mechanistic causes of APOs are still unclear.

Placentation plays a pivotal role in the health of the fetus and the pregnant person. While critical placentation events occurring in the first few weeks lay the foundation for a successful pregnancy, the placenta continues to adapt across gestation in response to a variety of maternal factors and external stimuli.[18] However, this process can become impaired, leading to morphological changes that decrease the efficiency of nutrient delivery and endocrine signaling.[17] Of particular note, reductions in angiogenesis within the villous vascular tree are associated with poor maternal and fetal outcomes such as preeclampsia and intrauterine growth restriction.[19,20] Fetal vascular percentage (FV%), or the proportion of the villous space occupied by fetal capillaries, is reduced in placentas from people who experience an APO.[21] Yet, mechanistic causes of these reductions are not fully understood.

Physical activity is a modifiable lifestyle factor with known systemic vascular benefits that are apparent across the lifespan. Higher weekly minutes of physical activity are associated with lower arterial stiffness[22] and improved endothelium-dependent vasodilation.[23] Mechanistically, the laminar shear stress induced by increased blood flow during exercise engenders positive adaptations to the endothelium such as enhanced nitric oxide release and bioavailability; these

adaptations occur at both the local level of the exercising limb and in the non-exercising vasculature.[24] At the same time, physical activity upregulates angiogenic factors through the activation of vascular endothelial growth factor (VEGF) genes.[25,26] Exposure to both acute bouts of exercise and long-term aerobic training increase serum VEGF, indicating increased angiogenesis due to activity.[27] These adaptations to physical activity improve blood flow and nutrient delivery throughout the body.

Sedentary behavior, or time spent seated or supine with low energy expenditure, is emerging as a risk factor for chronic diseases that is distinct from insufficient physical activity.[28,29] For example, a desk worker with high levels of sedentary behavior may also achieve high levels of physical activity by completing a run every day after work. Similar to low physical activity, high sedentary behavior is also distinctly correlated with poor vascular health outcomes. Greater sedentary behavior is associated with increased blood pressure and arterial stiffness[22,30], and higher sedentary time has been associated with increased incident cardiovascular disease 2-15 years later in a broad range of study populations.[29]

In pregnancy, there is emerging evidence that patterns of physical activity and sedentary time are associated with maternal and fetal health outcomes such as gestational diabetes mellitus, preeclampsia, and birth weight.[31,32] Research on physical activity and sedentary behavior among pregnant people has largely utilized less valid self-report questionnaires, but an initial study in 100 participants using state-of-the-art objective monitoring indicated that these behaviors vary widely across individuals and are linked to maternal-fetal health outcomes.[33,34] Those with high sedentary behavior across pregnancy (~11 h/day) had a nearly 4-fold increase in hypertensive disorders of pregnancy compared to participants with the least sedentary behavior (~8 h/day).[35] Similarly, those with higher vs. lower sedentary behavior bore infants with impaired fetal growth as indicated by

significantly lower ponderal index.[33] The mechanisms by which these relationships operate, however, have not been rigorously studied.

In addition to activity profile, other aspects of maternal cardiovascular health prior to and during pregnancy are also linked to APOs. These relationships can be bidirectional, whereby poor cardiovascular profile at the outset of pregnancy can increase the risk of certain APOs (e.g., preeclampsia and gestational diabetes), but the occurrence of an APO in a previously healthy person also appears to increase future risk of diseases such as high blood pressure or diabetes.[2–4] Preeclampsia in pregnancy increases the mother's lifetime risk of dying of heart disease or stroke by 71%.[36] While blood pressure typically returns to normal values following delivery of a pregnancy complicated by preeclampsia or gestational hypertension, many women go on to experience an increase in blood pressure over the next several years.[37] Whether pregnancy operates as a "stress test" that unmasks underlying predisposition to heart disease, or rather that the pathophysiology of hypertension or other APOs in pregnancy changes the maternal systemic vascular environment via sustained upregulation of maladaptive signaling from the placenta, is still not fully understood. Either way, understanding mechanisms and intervention targets for reducing APOs is critical to improve short- and long-term maternal health.

The American Heart Association's "Life's Essential 8" is a metric of cardiovascular health comprised of eight components: physical activity, blood pressure, cholesterol, blood glucose, body mass index (BMI), diet, sleep, and smoking. Ideal health across these components predicts a reduced risk of long-term cardiovascular disease.[38,39] This metric can be adapted to provide a tool for assessing cardiovascular health (prior to the development of disease), with higher scores in the components being averaged to yield an increased composite score that indicates better overall cardiovascular health. Life's Essential 8 was released in 2022 and has yet to be applied to

pregnancy, but the prior iteration ("Life's Simple 7", which did not include sleep) has been associated with APOs.[12] No research has examined how these components relate to placental vascular development. Examining placental vascular morphology as it relates to markers of maternal cardiovascular health during pregnancy can elucidate these associations and inform additional potential intervention targets.

Understanding whether physical activity, sedentary behavior, and maternal cardiovascular health during pregnancy impact villous vasculature may shine light on the mechanistic pathway through which APOs develop and contribute to future disease risk. Given the known vascular adaptations associated with physical activity and sedentary behavior in the rest of the body, and the observed associations between maternal cardiovascular health and APOs, there is strong rationale to hypothesize that low physical activity, high sedentary behavior, and poor maternal cardiovascular profile are associated with poor vascularization in the placenta – yet, this is unstudied. Figure 1 displays the conceptual framework for the proposed study. Relationships between activity profile, maternal cardiovascular health, and APOs are well-established in the literature, as are the relationships of placental health with APOs as well as APOs with future maternal cardiovascular disease (solid arrows). The proposed aims will investigate the largely unstudied research questions regarding whether activity profile and maternal cardiovascular health impact placental health (open arrows) in a way that may relate to the occurrence or prevention of adverse pregnancy outcomes.

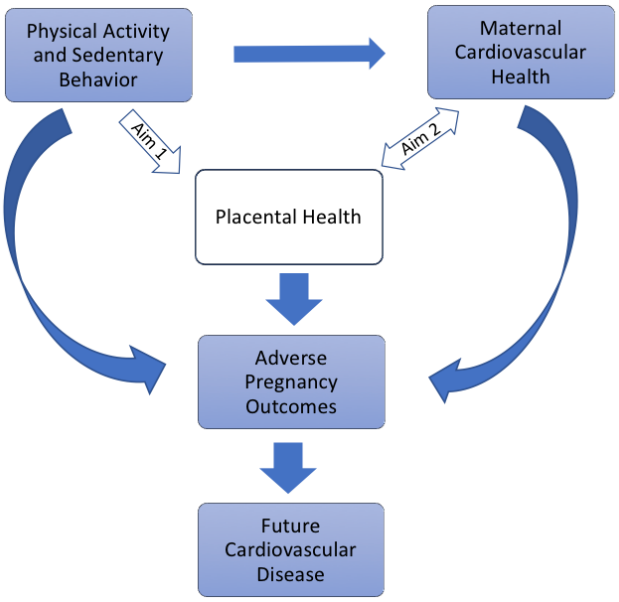


Figure 1. Conceptual Framework

1.3 Specific Aims

Specific Aim 1: To investigate the relationship between activity profile, both across pregnancy and by trimester, on placental villous vascular development.

Hypothesis: Higher physical activity and lower sedentary behavior will be associated with higher FV%.

Specific Aim 2: To investigate the relationship between maternal cardiovascular health and placental villous vascular development.

Hypotheses: Higher maternal cardiovascular health summary score and higher component scores will be associated with higher FV%.

2.0 Review of Literature

2.1 Cardiovascular Disease Burden among Women

Cardiovascular disease is the leading cause of death in both males and females, but distinctive patterns emerge between the sexes with regards to presentation, age of diagnosis, and risk factors.[40] While risk factors such as smoking, physical inactivity, hyperlipidemia, and metabolic disease are common to both sexes, contributors including the reduction of estrogen during menopause and pregnancy complications such as hypertensive disorders of pregnancy or gestational diabetes are unique to females.[41] However, the majority of research in cardiovascular disease has either only investigated male participants or has not stratified analyses by sex. Thus, female-specific data on causes, experiences, and treatments of cardiovascular disease are lacking.

2.1.1 Recommendations for Optimal Cardiovascular Health

Primordial prevention of cardiovascular disease, i.e., preventing the incidence of risk factors for cardiovascular disease, is now recognized as a critical strategy for reducing the burden of cardiovascular morbidity and mortality across the population.[9] Yet, maintaining cardiovascular health across the lifespan is a challenge, and causes of cardiovascular risk factors and disease are complex. Factors affecting vascular function, such as blood lipids, blood glucose, and endothelial function all contribute to how well or poorly the cardiovascular system ages.[42] As such, the American Heart Association has set standards for several key components of "ideal"

cardiovascular health.[9] Coined "Life's Essential 8", these components comprise eight modifiable risk factors for cardiovascular disease including blood pressure, total cholesterol, fasting blood glucose, physical activity, sleep, diet, body mass index (BMI), and smoking. Recommendations for ideal health goals for adults are presented in Table 1.[38]

Table 1. The American Heart Association's Life's Essential 8

Cardiovascular Health Component	Ideal Health Definition
Smoking	Never smoker
BMI	<25 kg/m^2
Blood pressure	<120/<80 mm Hg
Fasting plasma glucose	<100 mg/dL or HbA1c<5.7
Blood lipids	<130 mg/dL non-HDL cholesterol
Physical activity	≥ 150 min/week moderate intensity or ≥ 75 min/week vigorous intensity, or equivalent combination
Sleep	7-<9 hours of sleep per night
Healthy diet	≥15 points on the Mediterranean Eating Pattern for Americans questionnaire or ≥ 95th percentile on DASH-style diet or the Healthy Eating Index (adequate fruits and vegetables, nuts, low-fat dairy, fish, and whole grains; sufficiently low sodium, red or processed meats, and sweets)

Ideal cardiovascular health in most or all of these components has been associated with marked reductions in the incidence of subclinical and overt cardiovascular disease and mortality.[43–45] Risk reduction for those with the highest number of ideal health metrics compared to those with the fewest is profound: risk of overall cardiovascular disease is 80% reduced in the healthiest groups, and the risk of cardiovascular mortality is reduced by 75%.[43] Furthermore, maintaining a higher score of cardiovascular health over several years during midlife is associated with a 27% reduction in the incidence of cardiovascular disease.[46] A higher ideal cardiovascular health score is associated with reduced incidence of heart failure[46] and improved markers of left ventricular

function such as left ventricular mass and wall thickness.[47] However, fewer than 20% of adults in the United States achieve ideal cardiovascular health scores and disparities exist between males and females.[39]

2.1.2 Causes of Sex-Specific Cardiovascular Disease

The female experience of cardiovascular disease is distinct from that of males. While incidence of cardiovascular disease in men is high at younger ages, once past menopause the rate in females matches or exceeds that of males.[48] Moreover, symptoms and clinical presentations of cardiovascular disease are different in males and females.[40] Females share the same risk factors for cardiovascular disease as men, such as smoking, diabetes, and physical inactivity, but are also subject to several sex-specific risk factors. These include autoimmune disorders that affect a disproportionate number of females, but also hormonal risk factors such as early menarche (onset of menses), menopause, polycystic ovarian syndrome, and APOs.[41,48] As such, childbearing is a critical period during which to investigate cardiovascular disease risks and progression.

2.2 Placental Physiology

The placenta is a transient organ, but plays an essential role in both the development of the fetus and the health of the mother.[49] Its primary function is to provide an interface between maternal and fetal blood, allowing nutrient and gas exchange to occur while protecting the fetus from exposure to teratogenic or other harmful substances. It also acts as an endocrine organ, secreting hormones essential to the maintenance of the pregnancy.[49] The placental structure

undergoes substantial changes across gestation to support the developing fetus. If this development is inhibited or maladaptive, it can lead to negative health consequences for the mother and fetus.

2.2.1 Placental Structure and Development

The macroscopic structure of the placenta can be rudimentarily distilled into the structures of the maternal surface, the structures of the fetal surface, and the intermediate space between the maternal and fetal sides (Figure 2[50]). For the purposes of this review and the proposed project, the intermediate space and the adaptations to the interface between the uterine structures and the placenta will be considered.

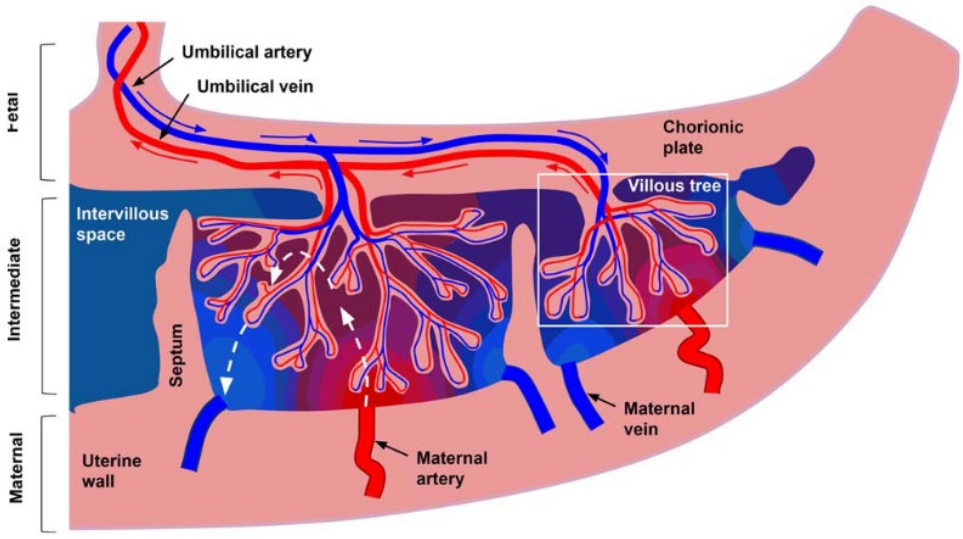


Figure 2. Macroscopic Schematic of the Placenta

The villi are the functional units of the placenta. Villous trees develop across gestation, surrounded by a continuous multinucleated syncytiotrophoblast layer and a layer of cytotrophoblast epithelium; these villi contain fetal vasculature.[49] Maternal blood enters the

intervillous space and bathes the villi, allowing for oxygen, nutrient, and waste product exchange while the trophoblast bilayer maintains a barrier between maternal and fetal circulation. However, this free flow of maternal blood into the intervillous space does not occur right away. Rather, several developmental steps must take place before the intervillous space is inundated with maternal blood.

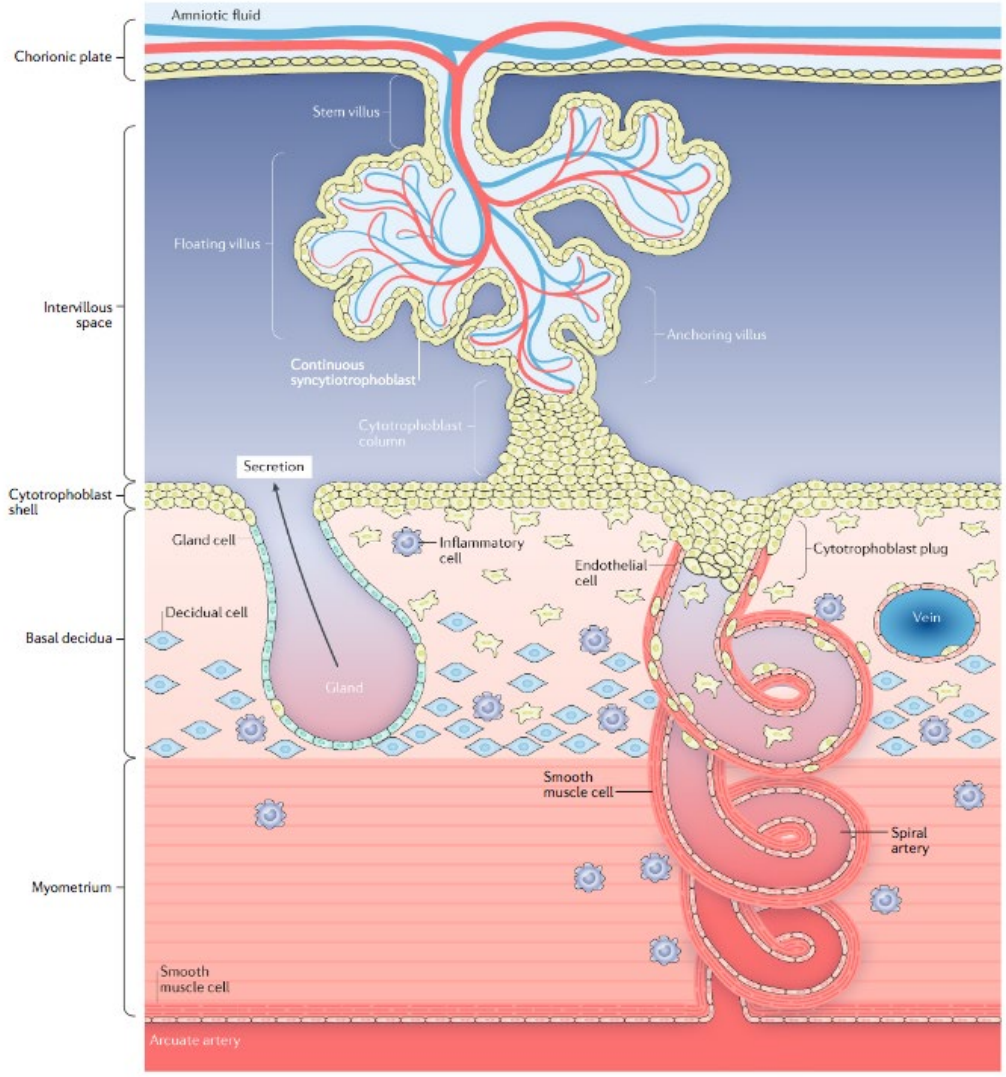


Figure 3. Trophoblast Plug of the Uterine Spiral Artery

Following implantation of the blastocyst in early development, trophoblast cells migrate into the decidua of the uterine wall and invade the structures within.[17,51,52] Invasion of the spiral arteries by endovascular trophoblast cells leads to remodeling of these arteries; endothelial and smooth muscle cells in the walls of the spiral arteries are replaced by fibrinoid matrix and trophoblast cells.[19] This leads to a widening of the vessel lumen, allowing for a low-resistance flow of maternal blood into the intervillous space. However, the spiral artery lumen are occluded by trophoblast plugs in the early weeks of pregnancy (Figure 3[19]). This yields a low-oxygen tension environment that ensures the villi develop with minimal exposure to turbulent blood flow or oxidative stress. As gestation progresses, the trophoblast plugs slowly compact and disperse, allowing for maternal erythrocytes to traverse into the intervillous space. Timing of plug dispersion is still a matter of debate, but it appears plug material remains well into the second trimester.[53]

Poor spiral artery remodeling, wherein the smooth muscle of the vessel wall remains, is a potential culprit in a number of negative pregnancy outcomes.[20] Retention of smooth muscle cells means the vessel wall remains sensitive to vasoactive substances, and artery diameter can become constricted. This leads to impaired or turbulent blood flow and can cause mechanical damage or oxidative stress to the villous structures.[19]

Assuming normal development occurs, the villi and fetal vessels within continue to branch and grow.[49] Fetal capillary development is sensitive to the presence or absence of oxygen and nutrients. In normal development, the villous vasculature undergoes a two-step process of increasing the vascular architecture and overall area.[49,54] Fetal vessels continuously develop throughout the pregnancy. In the second trimester, branching angiogenesis predominates while

oxygen concentration is low; this is driven by upregulation of vascular endothelial growth factor A (VEGF).[55] Capillaries increase in total number and branching architecture, which allows for more overall capillaries to interface with the maternal blood flow. In the third trimester, branching angiogenesis recedes and nonbranching angiogenesis dominates as oxygen concentration is higher and placental growth factor (PlGF) is upregulated.[55] Existing capillaries grow by lengthening and bulge against the epithelial bilayer separating them from the maternal blood to maximize exchange of metabolites.

As the placenta approaches parturition, signs of placental aging become more apparent. The nuclei of the continuous syncytiotrophoblast begin to aggregate, forming syncytial knots. These formations can be used to identify placentas that have begun to senesce before the pregnancy reaches term.[18] If maternal blood flow becomes compromised, villous adaptations such as advanced villous maturation and avascular villi will present.[20] These adaptations have been observed in the presence of APOs. However, whether the villous vascular development is sensitive to differences in maternal cardiovascular health and activity profiles is not yet clear. We hypothesize that villous vascular development, and thereby FV%, will be enhanced with higher levels of physical activity, lower levels of sedentary behavior, and more optimal markers of maternal cardiovascular health during pregnancy.

2.3 Adverse Pregnancy Outcomes, Maternal Cardiovascular Disease Risk, and Placental Findings

2.3.1 Adverse Pregnancy Outcomes and Future Maternal Cardiovascular Disease Risk

Risk of developing cardiovascular disease in the years following pregnancy is elevated in those who experience an adverse pregnancy outcome such as gestational hypertension, preeclampsia, preterm birth, or a growth-restricted fetus. These pregnancy outcomes are relatively common in the United States, with hypertensive disorders of pregnancy (gestational hypertension and preeclampsia) occurring in 5-15% of pregnancies[56,57], and the prevalence of preterm birth and fetal growth restriction both at 10%.[58,59] Moreover, these rates have been steadily increasing over the last several decades, with nearly twice as many diagnoses of hypertensive disorders of pregnancy per year in 2014 than in 1993.[60] The immediate health effects of these disorders can be quite detrimental to both mother and child, but it is becoming increasingly clear that long-term consequences are also a major concern. In particular, experiencing an APO places the mother at an increased risk of developing cardiovascular disease later in life.[2,5]

In individuals who experience hypertensive disorders of pregnancy, the overall risk of developing any cardiovascular disease is double that of individuals who only experience normotensive pregnancies; the risk of heart failure alone is 4.2 times higher in those who had preeclampsia in pregnancy compared to those with normotensive pregnancies.[5] While chronic hypertension and heart failure can develop across the lifespan following hypertension in pregnancy, the risk is greatest within 5 years of delivery.[56,61] Subclinical measures of cardiovascular disease such as carotid intima-media thickness and atherogenic lipid profile show deleterious changes within 10 years of a preterm birth[62], and hypertension often develops in the

decades following a preterm delivery.[63] Hypertensive disorders of pregnancy and low birth weight increase the odds of atherosclerotic cardiovascular disease by 27% and 12% respectively[64], and a history of preterm birth increases the risk of cardiovascular disease by a factor of two.[5] A person with a history of preeclampsia has a 71% greater risk of dying of heart disease or stroke over their lifetime[36], and a 20% increased risk of dying before the age of 70.[57] Given these data, the American Heart Association has designated APOs as major risk factors for cardiovascular disease and has called for comprehensive specialized medical management of all women with cardiovascular disease presentations before, during, or after pregnancy.[36]

2.3.1.1 Physiological Basis for Relationship between Adverse Pregnancy Outcomes and Future Cardiovascular Disease

A number of factors contribute to the relationship between adverse pregnancy outcomes and cardiovascular disease. Risk factors and etiologies common to both APOs and cardiovascular disease indicate that APOs may be an early presentation of previously subclinical disease, which manifests as overt disease in the years following the pregnancy.[6] Similar pathologies, such as atherosclerotic-type lesions, are found in both outcomes.[5] However, evidence also exists to suggest that pathophysiological processes that begin during pregnancy continue postpartum and contribute to the development of disease.[5,7] While "primordial prevention" strategies – aimed at preventing the development of risk factors for cardiovascular disease – would benefit from a clear understanding of whether adverse pregnancy outcomes unmask subclinical disease or cause it, it is likely that these two explanations are not mutually exclusive.[8]

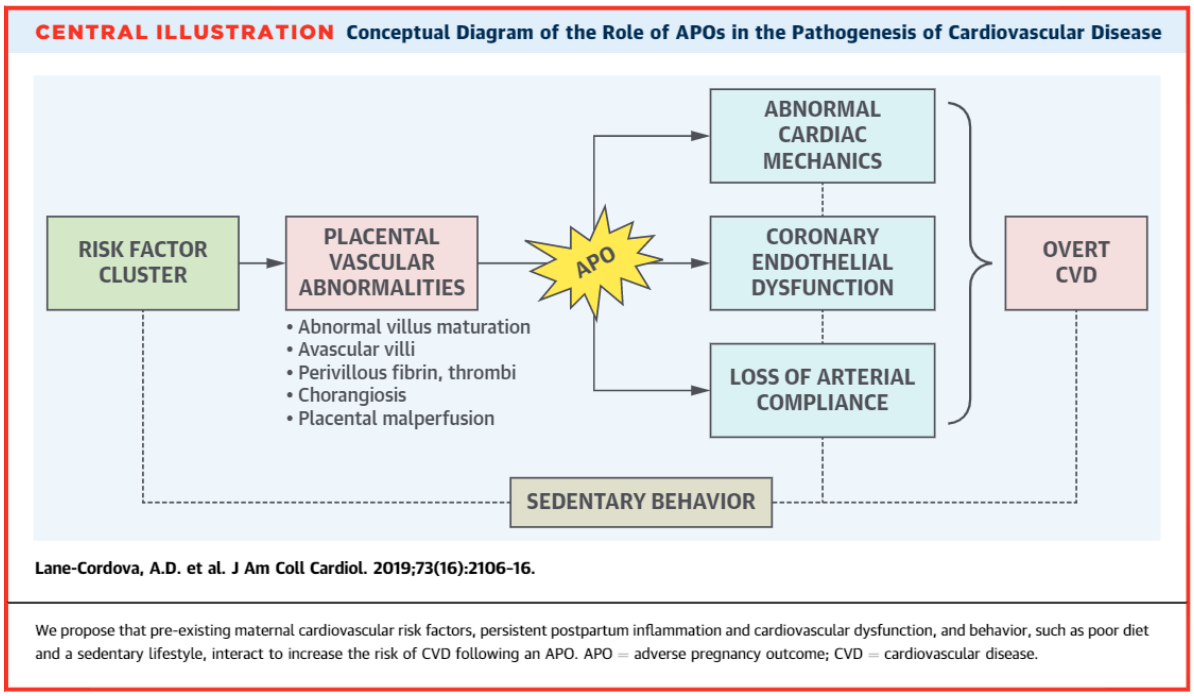


Figure 4. Conceptual Relationship Between Adverse Pregnancy Outcomes and Cardiovascular Disease

Given the complex relationship between cardiovascular disease risk factors, APOs, and overt clinical disease, as demonstrated by Lane-Cordova, et al[2] (Figure 4), it is helpful to consider both the ways in which risk factors influence the development of APOs and how APOs can modify the maternal systemic environment in ways that lead to new disease or accelerate existing disease processes. In the case of the former, people who enter pregnancy with risk factors for cardiovascular disease such as chronic hypertension, pre-gestational diabetes mellitus, obesity, inadequate activity, and kidney disease are at increased risk for developing APOs.[65] As demonstrated by Lane-Cordova, et al[2] in Figure 5, these factors can contribute to inadequate placentation and subsequent ischemia.

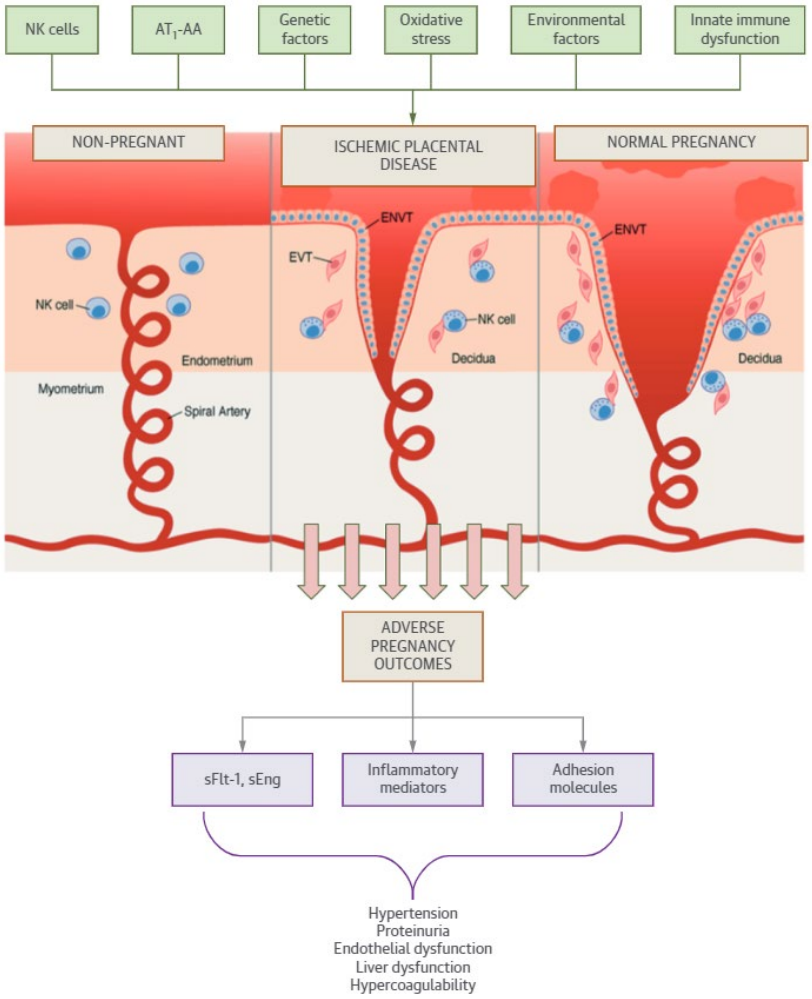


Figure 5. Pathogenesis of Adverse Pregnancy Outcomes and Subsequent Cardiovascular Disease

Adverse pregnancy outcomes, particularly hypertensive disorders of pregnancy, present with maternal systemic upregulation of inflammatory pathways and oxidative stress, which lead to the endothelial dysfunction common to hypertension and cardiovascular disease states.[2,66] This occurs due to increased oxidative damage in the placenta, which releases pro-inflammatory cytokines into maternal circulation.[67]

The systemic pro-inflammatory, pro-coagulatory upregulation can persist after delivery, contributing to the increased risk for cardiovascular disease after exposure to a hypertensive disorder of pregnancy.[4] Endothelium-dependent vasodilation continues to be impaired in the 12

months following preeclampsia in spite of the resolution of clinical symptoms, and pre-pregnancy cardiovascular risk factor status does not explain this phenomenon.[7] In addition, the cardiac remodeling and vascular dysfunction that are seen in hypertensive disorders of pregnancy can persist postpartum[68], which is likely due in part to a continued upregulation of markers of endothelial dysfunction leading to increased afterload and hypertension.[2] While it is still uncertain whether these changes are induced *de novo* by the poor placentation common to APOs or are simply an acceleration of a pathophysiology that was present prior to pregnancy, it is clear a poorer cardiovascular profile following exposure to an APO is a common outcome.

2.3.2 Placental Findings in Adverse Pregnancy Outcomes

Lesions in the placenta are common to every class of adverse pregnancy outcome.[49] The specific pathology varies depending on the outcome, but some types of lesions are present across several APOs. Several lesions are directly related to poor spiral artery remodeling, while others are downstream effects of the ischemia and damage induced by poor remodeling.

2.3.2.1 Maternal Vascular Malperfusion Lesions

Maternal vascular malperfusion (MVM) lesions are a class of pathologies that manifest on the decidual side of the placenta. These can include poor remodeling of spiral arteries that leads to decidual vasculopathy such as fibrinoid necrosis or atherosis[20]; these lesions are similar to atherosclerotic plaques seen elsewhere in the vasculature. Additional lesions can include downstream villous changes such as infarcts, hypoplasia, accelerated villous maturation, or retroplacental hematoma.[69] As with APOs, it is unclear whether these pathologies come about due to an underlying maternal condition that predisposes to cardiovascular disease or if the placental

pathology initiates a systemic physiological state that is generative of cardiovascular disease. However, some evidence indicates that MVM lesions are associated with increases in plasma soluble endoglin, a placental biomarker that negatively influences endothelial function.[70] MVM lesions are fairly uncommon in random samples of pregnant people, with an incidence rate of 8.4% in low-risk nulliparous women.[71] In women who develop MVM however, the incidence of APO is as high as 48-74%.[70,71] Further, while many women who demonstrate MVM pathology will also present with clinical APOs, not all do. In a sample of placenta tissue retrieved from uncomplicated pregnancies, 35.7% were found to have MVM lesions.[72] It is not clear why some women with MVM lesions are spared from APOs, highlighting the need for mechanistic research.

2.3.2.2 Villous Space and Fetal Placental Vascular Findings

As discussed in section 2.2.1, villous and vascular architecture develop in a biphasic manner across gestation. Branching development predominates early on in the presence of lower oxygen concentration, while non-branching growth occurs later in gestation as oxygen concentration in the intervillous space increases.[54,55] In cases of adverse pregnancy outcomes, this development is impaired, leading to poor perfusion of the fetal compartment and negative health outcomes. Depending on the timing within the disease course of when the placental tissue is analyzed, this may manifest as decreases (avascular villi due to infarction or fetal thromboses) or increases in capillarization within the villi (congestion as an adaptation to poor maternal perfusion, or resulting from neighboring avascular villi).[73] Reductions in fetal capillary outcomes are seen in many APOs, but are particularly evident in intrauterine growth restriction with or without preeclampsia.[21,74–76] Preeclampsia demonstrates villous parameter changes, but changes in vascular outcomes may be restricted to preeclampsia that onsets before 34 weeks of

gestation.[21,74,77] As such, understanding what risk factors contribute to the development of disease, including disease severity, is a critical research gap.

2.3.2.3 Measurement of Placental Pathology

Placental health is characterized in a number of ways. Gross pathological examination is typically performed first, including placental weighing and inspection for grossly visible lesions. These may include areas of infarction, fetal vascular thromboses, areas of hemorrhage, maternal surface clots/hematomas, and grossly visible villous lesions.[18] Weight of the placenta can be used in a ratio with newborn birth weight as an indirect measure of placental efficiency[78,79]; if the placenta is small compared to the birthweight, it indicates the placenta was highly efficient at delivering nutrients to the growing fetus. Other gross lesions such as infarctions or hematomas can indicate that pathological processes were present in the uterine decidua, which may have impacted nutrient exchange with the fetus.[49]

Following a macroscopic evaluation, microscopic imaging can be used to examine the decidual and villous tissues. Histology, particularly staining with hematoxylin and eosin, is used to determine if MVM lesions are present.[18] These stains can detect MVM lesions including decidual vasculopathies such as atherosis and fibrinoid necrosis, as well as villous damage such as accelerated villous maturation or distal villous hypoplasia.[20] These lesions are typically characterized as present or absent, but are not quantified in a continuous fashion.

Immunohistochemistry staining is used to highlight cell types such as endothelial and trophoblast cells.[13,18] This type of analysis can be used to directly measure the area or volume of the villous space, including fetal capillary density. This allows for the villi to be characterized continuously, allowing for more precise quantification of vascular development and greater statistical power when examining outcomes. While this type of investigation is not new[13,14], recent

advances in computer-assisted analysis have reduced barriers to its use in research.[80] As such, it is possible to examine how differences in villous architecture, including FV%, are related to differences in maternal cardiovascular health characteristics. We plan to use this method in the proposed project to measure FV% and study its associations with physical activity, sedentary behavior, and maternal cardiovascular health.

2.4 Impact of Physical Activity and Sedentary Behavior upon Health Outcomes in Pregnancy

2.4.1 Recommendations for Physical Activity and Sedentary Behavior During Pregnancy

Guidelines for participation in physical activity for adults have been published by a number of organizations and governmental bodies. In short, adults are encouraged to accumulate at least 150 minutes per week of moderate-intensity physical activity (above 3 metabolic equivalents, or METS) or 75 minutes per week of vigorous-intensity physical activity (greater than 6 METS).[81–83] This guideline is accompanied by the recommendation to engage in physical activity on most or all days of the week, but that any activity is better than no activity. Achieving the recommended volume of physical activity is associated with reduced risk for many adverse health outcomes including all-cause mortality, cardiovascular disease, cognitive decline, and mood disorders such as depression and anxiety.[81]

Historically, recommendations for engaging in physical activity during pregnancy came with a number of stipulations and caveats. As recently as 1985, guidance issued by the American College of Obstetricians and Gynecologists (ACOG) stated pregnant women should only exercise

for 15 minutes at a time and should keep their heart rate below 140 beats per minute. After several research studies demonstrated the safety of participating in physical activity during uncomplicated pregnancies, these restrictions were removed. Further evidence showed that participation in regular physical activity was beneficial during pregnancy, and the current (2020) ACOG opinion encourages aerobic and resistance activity during pregnancy.[84]

Health and fitness organizations such as the American College of Sports Medicine and the Canadian Society for Exercise Physiologists extend the guidance for physical activity for adults to pregnant people in most circumstances[85,86]; this guidance is mirrored by the United States Department for Health and Human Services[81] and the World Health Organization.[83] As such, it is recommended that pregnant people engage in 150 minutes per week of moderate intensity physical activity, accumulated over most days of the week, and account for training status when considering whether vigorous activity is appropriate. Exercise is only contraindicated in the case of certain pregnancy complications such as premature rupture of membranes, preeclampsia, and placenta previa.[86]

Specific guidelines for sedentary behavior during pregnancy have not been published by any organization or governmental body to date. The World Health Organization included a recommendation to reduce sedentary time in its 2020 guidelines, but noted that this was not based on specific research in pregnancy but was rather an extrapolation of the guidance for all adults.[83] Moreover, the guidance made clear that while reducing sedentary time is beneficial, the research currently does not support a specific threshold of sedentary time. Even in general populations, very few specific guidelines for sedentary behavior exist. The notable exception is the Canadian 24-hour movement guidelines for adults, which recommend limiting sedentary time to less than 8

hours per day and breaking up prolonged sitting as often as possible.[82] However, these guidelines are for all adults, not specifically pregnant people.

2.4.2 Physiological Basis for Physical Activity and Sedentary Behavior Guidelines

The endothelium plays a complex role in the vasoconstriction or vasodilation of the blood vessel walls, the initiation of inflammatory cascades, and blood coagulation.[87,88] Dysfunction of the endothelium is related to development of atherosclerotic plaques, as leaky vessel walls can lead to invasion by macrophages that in turn become inundated by oxidized low-density lipoprotein molecules. This initiates a series of events that can result in the development of a plaque lesion.[87] In addition to atherosclerosis, endothelial dysfunction – particularly relating to the maintenance of vascular tone – can affect blood pressure. The endothelium is responsible for the synthesis and release of nitric oxide (NO), which is a potent vasodilator working on the smooth muscle cells of the vasculature. Dysfunction occurs when an imbalance exists between nitric oxide production and consumption.[87] When the vessels do not dilate appropriately, there is an increase in shear stress on the vessel walls. This can result in a dysfunctional loop of vasoconstriction and vessel damage, which can in turn initiate the atherosclerotic process. High arterial pressures also contribute to remodeling of cardiac structures, which is related to heart failure incidence and progression.[89]

Physical activity has a variety of mechanisms by which it reduces the risk of cardiovascular disease, including the improvement of endothelial function, blood pressure, and lipid profile. The laminar shear stress associated with increased blood flow caused by aerobic exercise leads to an increase in NO production and bioavailability, can increase angiogenesis, and improve antioxidant profile.[88,90] People who are habitually active demonstrate improved markers of endothelial

function compared to those who are inactive[88], and aerobic exercise training improves endothelial function in previously untrained individuals.[91] Furthermore, angiogenesis and remodeling of the conduit arteries is a well-established adaptation to exercise training[24]; this increase in the capacity of the vasculature to deliver nutrients is not limited to the exercising limbs.[27] This allows for the entire body to reap the vascular rewards of participating in physical activity.

Risk of cardiovascular disease or cardiac events decreases as activity levels increase in adults.[10,92] Of note, there is evidence that the greatest gradient of improvements can be seen when individuals move from no activity to some activity.[93] Relevant to studying women during and immediately after childbearing years, women who initiate an active lifestyle in midlife demonstrate improved cardiovascular outcomes compared to their peers who remain sedentary.[94] Given this evidence, physical activity is recommended as a means of primary and secondary prevention for cardiovascular disease.[95]

Mechanisms linking sedentary behavior, independent of physical activity, to cardiovascular outcomes are still being elucidated. However, hypotheses include that prolonged sedentary behavior reduces blood flow and subsequent laminar shear stress, reduced metabolic demand, and decreased lipid metabolism.[28] Endothelial function appears to be reduced after prolonged bouts of sitting[96], and more sedentary behavior is associated with increases in blood pressure.[30,97] In observational studies, sitting time is associated with increased rates of cardiovascular disease and all-cause mortality, and these rates are further increased in populations with high sedentary behavior in combination with chronic disease.[29]

2.4.3 Physical Activity and Sedentary Behavior Epidemiology in Pregnancy

Very few studies have examined the prevalence of meeting the guidelines for recommended physical activity during pregnancy. However, it appears that the majority of pregnant people do not achieve 150 minutes per week of moderate intensity physical activity; rates among various cohorts range from 15-35% achieving this threshold.[35,98–101] Sedentary behavior appears to also be high during pregnancy, comprising about two thirds of the waking day and with 80% of participants exhibiting more than 8 hours per day of sedentary behavior in two cohort studies.[33,35,102] It also appears that trajectories of physical activity and sedentary behavior are fairly stable across pregnancy.[35,98,101] While much more research is required to understand activity patterns in pregnant people, the existing data support the notion that pregnancy is a time with a relatively low-active, high-sedentary activity profile.

2.4.4 Impact of Physical Activity and Sedentary Behavior on Maternal Health Outcomes in Pregnancy

Benefits of physical activity during pregnancy for the mother include reductions in the risk of developing gestational diabetes, gestational hypertension, and preeclampsia. In the most comprehensive analysis to date, a 2018 meta-analysis[11] found that participation in physical activity during pregnancy reduced the odds of gestational diabetes mellitus by 38%, gestational hypertension by 39%, and preeclampsia by 41%, as compared to no-exercise controls. These benefits were attenuated when the analyses included studies that employed lifestyle behavioral interventions with unsupervised exercise and low rates of intervention uptake, indicating that participation in exercise is more effective if high adherence to the exercise prescription is achieved.

Additionally, there is evidence that participation in physical activity during pregnancy can attenuate gestational weight gain in participants who entered pregnancy with overweight or obesity[103], and that physical activity can reduce cesarean and other assisted delivery techniques in women across the BMI spectrum.[104] Finally, limited evidence indicates that peripartum anxiety and depression can be attenuated by inclusion of physical activity during pregnancy.[105,106]

2.4.5 Impact of Physical Activity on Placental Outcomes

During an acute bout of exercise, blood flow is shunted away from the viscera and towards the exercising musculature. As hypothesized by Bhattacharjee, et al[15] (Figure 6), this temporary reduction in blood flow may lead to positive adaptations in the placenta. Evidence in animal models indicates angiogenic factors are upregulated in the maternal circulation as a result of exercise training, which may indicate a mechanism for these adaptations.[107,108] Expression of angiogenic factor mRNA and protein expression in placental tissue is sensitive to physical activity in humans as well. Light and moderate intensity physical activity during pregnancy is related to an increased expression of angiogenin in the placenta, without evidence of increased oxidative stress[109], and more than 150 minutes per week of moderate-to-vigorous intensity physical activity (MVPA) in the 2nd trimester is related to increased placental expression of VEGF and its receptor VEGFR-1 in placenta tissue.[110] Furthermore, markers of oxidative stress were negatively associated with MVPA in pregnant participants with obesity, whereby more MVPA was associated with lower levels of oxidative stress..[111] Gross morphology may be related to activity levels as well: placental weight was lower with increased levels of MVPA in placentas with available pathology reports.[112] When measuring placental vascular development directly, absolute and relative villous vascular volume has been shown to be higher in pregnant runners compared to

inactive pregnant controls; evidence of increased mitotic activity and cell proliferation was also present, indicating the runners' placentas were still undergoing development at term.[13] Participants who continued to exercise during pregnancy had placentas with greater vascular volume than either those who ceased to exercise halfway through pregnancy or upon conception, indicating exercise during pregnancy itself may be a contributing factor.[14] Finally, a recent systematic review determined that while exercise had no effect on placental weight or placenta-to-birthweight ratio, favorable adaptations at the level of the villous tissue existed in exercise groups compared to non-exercising controls.[79] However, the number of studies included was relatively small and a great deal still remains to be uncovered regarding the impact of activity profile upon placental outcomes.

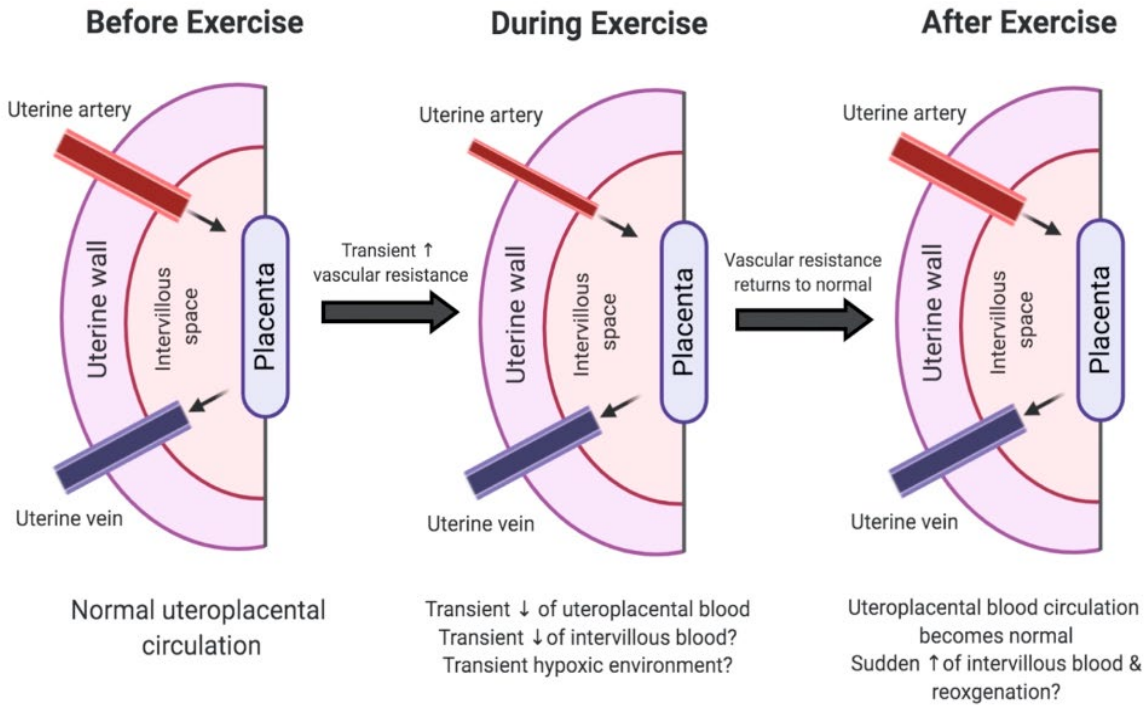


Figure 6. Hypothesized Effect of Exercise upon Placental Blood Flow

2.5 Impact of Maternal Cardiovascular Health Profile upon Health Outcomes in Pregnancy

2.5.1 Recommendations for Cardiovascular Health in Pregnancy

The Life's Essential 8 metrics of cardiovascular health put forward by the American Heart Association in 2022 have standardized recommendations for all adults.[38] These recommendations have eight components that are each scored on a scale of 0-100 and averaged to create a composite. In recognition that pregnancy is a unique time of increased healthcare utilization and that many people of childbearing age consider their obstetric provider to be their primary care provider, it is recommended that obstetricians and gynecologists be prepared to screen for Life's Essential 8 metrics in pregnancy and postpartum.[113] While specific guidelines for pregnant people have not been published, these metrics have been adapted for pregnancy for research purposes. Depending on the data available, these metrics typically adjust for physiological changes in BMI and fasting plasma glucose, or use alternative metrics such as pre-pregnancy BMI or the second trimester glucose screen.[114] Some differences are seen between pregnant and nonpregnant females in cardiovascular health scores. Perak, et al.[114] demonstrated certain factors are more or less likely to be categorized as less than ideal using the prior "Life's Simple 7" scoring configuration of "ideal", "intermediate", or "poor"[9] (Figure 7). During pregnancy, diet, BMI, and physical activity are particularly likely to be deficient, while smoking at fasting glucose are more likely to be ideal.

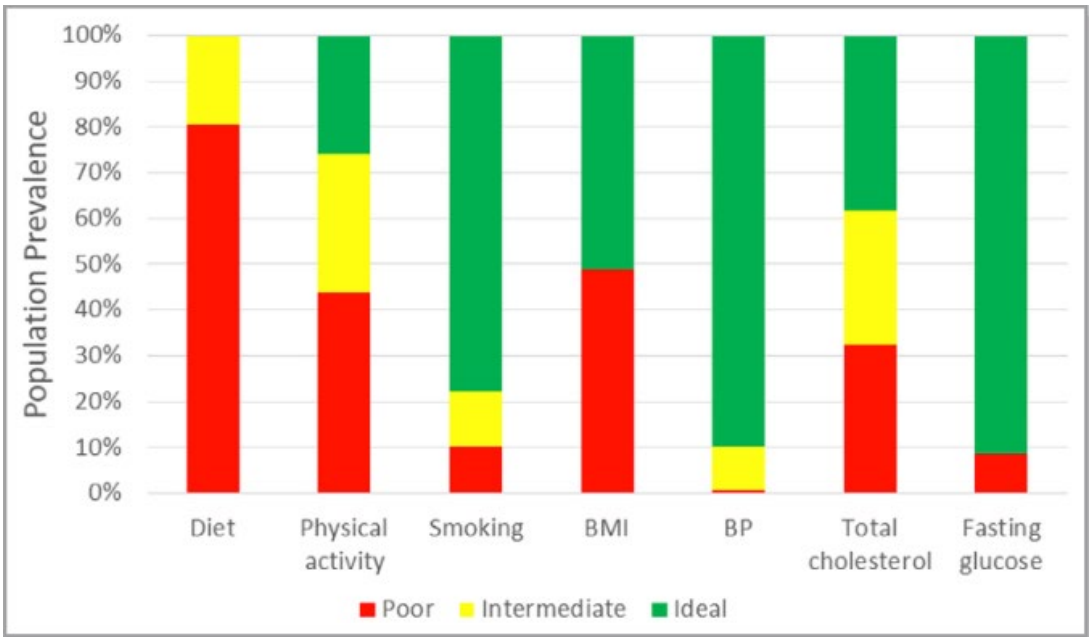


Figure 7. Prevalence of Cardiovascular Health Metrics in Pregnancy

2.5.2 Relationship of Life's Simple 7 Metrics in Pregnancy to Adverse Pregnancy Outcomes

Very few studies have examined the relationship between primordial prevention metrics of cardiovascular health and pregnancy outcomes. However, Perak, et al.[12] found that worse cardiovascular health score during pregnancy on a pregnancy-adapted Life's Simple 7 was associated with an increase in the relative risk of preeclampsia (Figure 8, far left columns). Presenting with more than two "poor" characteristics yielded a relative risk of 9.30 compared to presenting with all "ideal" metrics. Furthermore, it appears that rates of less-than-ideal cardiovascular health prior to pregnancy are increasing[115], and presenting with four "poor" cardiovascular health characteristics prior to pregnancy is associated with nearly 4 times the risk of preterm birth.[116]

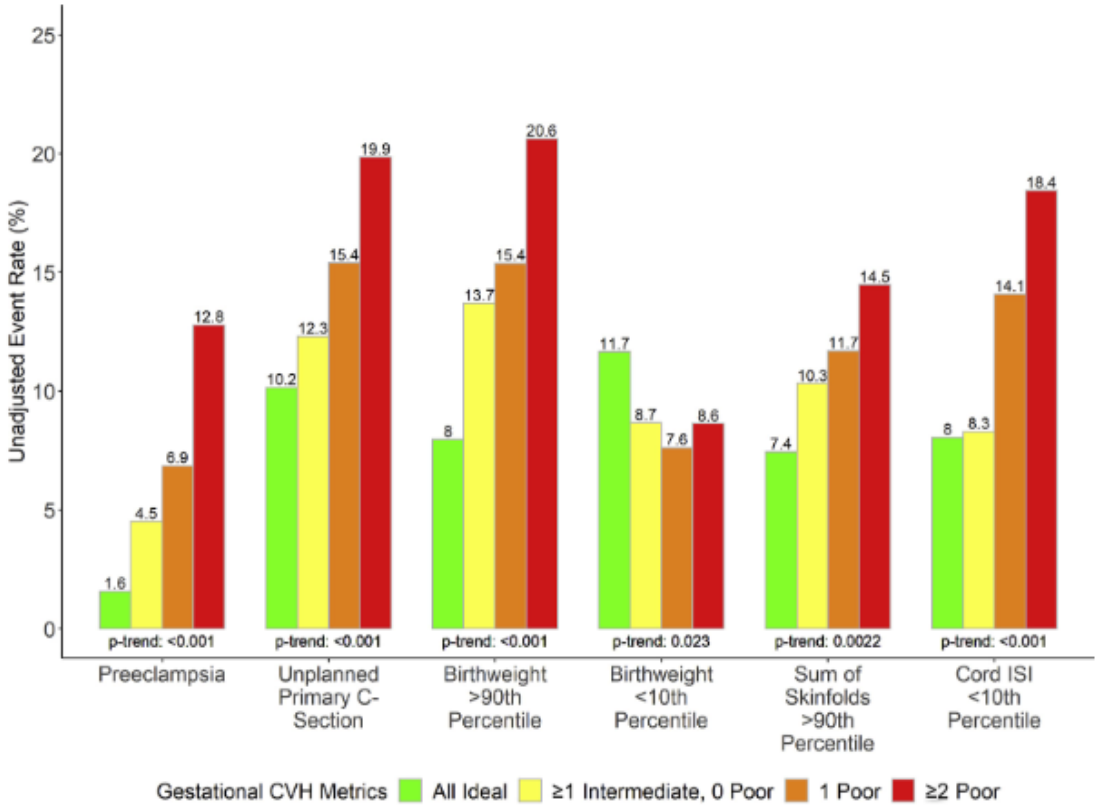


Figure 8. Rate of Adverse Pregnancy Outcomes per Number of Cardiovascular Health Metric Classifications

Given these relationships, is it logical to suspect that a composite cardiovascular health score during pregnancy would be associated with placental health. Some evidence suggests that pre-pregnancy and early pregnancy subclinical measures of cardiovascular disease are related to MVM lesions.[117] While relationships with component measures such as blood pressure and blood glucose are clear given these endpoints are markers of overt APOs[118,119], the framework of the Life's Essential 8 metrics have yet to be examined for their relationship to placental vasculature. Such findings would elucidate mechanisms through which these primordial prevention targets could reduce the risk of APOs, informing future intervention aims. We intend to address this important research gap in the proposed research.

2.6 Summary and Significance

Placental health is strongly related to the health of both mother and child, and this relationship carries over to long-term outcomes such as maternal cardiovascular disease. The factors affecting the development of the functional units of the placenta, the villi, are still being elucidated and could inform future preventative interventions. Modifiable risk factors for maternal cardiovascular disease, such as physical activity, sedentary behavior, and aspects of maternal cardiovascular health during pregnancy, are logical targets for such interventions, but how they impact placental vascular development has yet to be well characterized. Thus, this project aims to examine this research gap to better understand the mechanistic pathways through which maternal activity profile and cardiovascular health affect placental development, ultimately impacting maternal cardiovascular disease incidence in the years following pregnancy.

3.0 Methods

3.1 Experimental Design

Participants for this project were a subsample of a completed observational cohort study, the Monitoring Movement and Health Study (henceforth "MoM Health", n=37) and an ongoing multi-center observational cohort study, the Pregnancy 24/7 Study (henceforth "Pregnancy 24/7", n=29). In both studies, participants were recruited in their first trimester of pregnancy and followed through delivery in Pittsburgh, PA (MoM Health and Pittsburgh site of Pregnancy 24/7). The aim of MoM Health was to describe patterns of physical activity and sedentary behavior across pregnancy and associate these patterns with pregnancy and birth outcomes. MoM Health was funded by the American Heart Association (PI Gibbs, AHA 17GRNT3340016). MoM Health served as a pilot study for Pregnancy 24/7, and the data collection methods were nearly identical between studies. The primary aim of Pregnancy 24/7 is to associate trajectories of physical activity, sedentary behavior, and sleep (so-called "24-hour behaviors") across pregnancy with hypertensive disorders of pregnancy and other adverse pregnancy outcomes. Pregnancy 24/7 is funded by the National Heart, Lung, and Blood Institutes (PI Whitaker; R01 HL153095).

The subsample for this dissertation came from the full MoM Health cohort and Pittsburgh-area based Pregnancy 24/7 participants who co-enrolled in the Magee Obstetric Maternal & Infant (MOMI) Biobank. The MOMI Biobank acquires blood, urine, placenta, and cord blood tissue samples during pregnancy and at delivery. The MOMI Biobank is funded by the RK Mellon Foundation, the Clinical and Translational Science Institute (CTSI) at the University of Pittsburgh, and the Magee-Women's Research Institute (MWRI) and Foundation (PI Jeyabalan). Co-enrolled

MoM Health and Pregnancy 24/7 participants with successful placenta acquisition prior to July 1, 2022 constituted the sample for this study, which investigated the following specific aims:

Specific Aim 1: To investigate the relationship between activity profile, both across pregnancy and by trimester, on placental villous vascular development.

Hypothesis: Increased physical activity and decreased sedentary behavior will be associated with higher FV%.

Specific Aim 2: To investigate the relationship between maternal cardiovascular health and placental villous vascular development.

Hypotheses: Higher maternal cardiovascular health composite score and components will be associated with higher FV%.

3.2 Study Sample

3.2.1 Inclusion and Exclusion Criteria

Participants were eligible to enroll in MoM Health or Pregnancy 24/7 if they were 18-45 years of age, currently pregnant, and less than 13 weeks of gestation at consent. Participants were excluded from participation if they had chronic hypertension that was treated with antihypertensive medication, had pre-gestational Type I or Type II Diabetes Mellitus and were on medication to control blood sugar, were unable to walk ½ mile or climb 2 flights of stairs, or had some other serious medical condition that could confound the associations being studied.

MoM Health and Pregnancy 24/7 participants were eligible to co-enroll in the MOMI Biobank if they were planning to deliver at Magee-Women's Hospital of UPMC in Pittsburgh, Pennsylvania.

3.2.2 Recruitment

Recruitment methods were similar for MoM Health and Pregnancy 24/7. MoM Health recruitment took place during 2017-2018, while Pregnancy 24/7 began recruitment in 2020 and is planned to continue into 2023. Recruitment methods included physician referrals, flyer distribution, and online posting of the study screening hyperlink on the laboratory website, social media, and the "Pitt + Me" study recruitment database. In addition, recruitment emails were sent to participants who enrolled in the MOMI Biobank in their first trimester of pregnancy. Following completion of the online screening tool, eligible participants were contacted to schedule an orientation meeting and first trimester assessment visit. Assessments for MoM Health took place in person; assessments for Pregnancy 24/7 occurred in person or virtually via Zoom videoconferencing due to the SARS-CoV-2 pandemic.

Two pathways for co-enrollment in the MOMI Biobank were employed. In the first, participants were approached by MOMI Biobank staff at a clinical laboratory appointment. If not enrolled through this method, participants were approached at MoM Health or Pregnancy 24/7 second or third trimester study visits, and, if interested, enrolled.

3.3 Assessment Procedures

MoM Health and Pregnancy 24/7 participants completed assessments once during each trimester. First trimester assessments occurred between 8 weeks, 0 days (8.0) and 12 weeks, 6 days (12.6) of gestation. Second trimester assessments occurred between 20.0 and 22.6 weeks of gestation. Third trimester assessments occurred between 32.0 and 34.6 weeks of gestation. Gestational age was initially self-reported and was based on last menstrual period, ultrasound, and/or date of in vitro fertilization. Later, gestational age was abstracted from the medical record and retrospectively corrected if applicable.

3.3.1 Activity Profile

Physical activity and sedentary behavior were objectively measured for 1 week in each trimester (3 time points) using a thigh-mounted accelerometer (activPAL3, PAL Technologies, Glasgow, Scotland) as part of the MoM Health and Pregnancy 24/7 Studies. This is the best practice method for single-device, free-living assessment of both physical activity and sedentary behavior.[34,120,121] A standard, semi-automated approach where activity and posture are classified by proprietary software (PAL Technologies, Glasgow, Scotland) was used. Daily means of moderate-to-vigorous physical activity (MVPA) and sedentary behavior were calculated after removal of diary- and sleep actigraphy-informed non-wear and sleep periods.[122,123] Participant data was considered valid with ≥5 days of ≥10 hours of monitor wear time. MVPA and sedentary behavior were calculated within each day, averaged within each trimester, and then finally averaged across gestation as the mean of all measured trimesters.

3.3.2 Maternal Cardiovascular Health

Maternal cardiovascular profile was measured as a part of MoM Health and Pregnancy 24/7 and consisted of a composite score of the American Heart Association's "Life's Essential 8" factors[9], adapted for pregnancy and using available data from MoM Health, Pregnancy 24/7, and the electronic medical record. Adaptations to the general adult score included: 1) separate components for pre-pregnancy BMI and gestational weight gain; 2) use of the second trimester one-hour 50-gram oral glucose tolerance test rather than fasting glucose; and 3) omission of the cholesterol component due to unavailability of standard lipid assessment during pregnancy. Points were earned for each component and were scored on a scale of 0-100 according to the guidelines set forth by the American Heart Association[38] or, in the case of pregnancy-adapted components, clinically relevant cut points[124–127] (Table 2). A score of 100 corresponds to ideal cardiovascular health, while a score of 0 corresponds to the poorest cardiovascular health. The composite maternal cardiovascular health score was calculated by averaging the 8 component scores. Trimester-specific scores and overall gestational scores were computed. Up to three missing components were permitted in the calculation of the composite scores; if a component was missing, the average score of the remaining components comprised the composite score.

Blood pressure was abstracted from the medical record at each prenatal visit; as visit frequency increased with later gestation, blood pressures were averaged within trimester and then across gestation. Blood glucose was abstracted from the medical record as the 1-hour oral glucose tolerance test. Pre-pregnancy BMI was calculated as kilograms per meter squared (kg/m^2) using self-reported or measured height and self-reported pre-pregnancy weight.

Table 2. Maternal Cardiovascular Component Scoring

Component	Points	Level
Blood pressure	100	<120 mmHg SBP and <80 mmHg DBP
	75	120-129 mmHg SBP and <80 mmHg DBP
	50	130-139 mmHg SBP or 80-89 mmHg DBP
	25	140-159 mmHg SBP or 90-99 mmHg DBP
	0	≥160 mmHg SBP or ≥100 mmHg DBP
Blood glucose	100	< 135 mg/Dl
	50	135 – 199 mg/dL
	0	≥200 mg/dL
Pre-pregnancy BMI	100	< 25 kg/m^2
	70	25 - <30 kg/m^2
	30	30 - <35 kg/m^2
	15	35 - <40 kg/m^2
	0	≥40 kg/m^2
Gestational weight gain	100	Within IOM guidelines
	50	Outside IOM guidelines but within extended range
	0	Outside extended range
Diet quality score	100	8 points
	80	6-7 points
	50	4-5 points
	25	2-3 points
	0	0-1 points
Smoking status	100	Never
	50	Former
	0	Current
Physical activity	100	≥ 150 min/week
	90	120-149 min/week
	80	90-119 min/week
	60	60-89 min/week
	40	30-59 min/week
	20	1-29 min/week
	0	0 min/week
Sleep	100	7-<9 hours/night
	90	9-<10 hours/night
	70	6-<7 hours/night
	40	5-<6 or ≥ 10 hours/night
	20	4-<5 hours/night
	0	<4 hours/night

SBP=systolic blood pressure; DBP=diastolic blood pressure; IOM=Institute of Medicine

Gestational weight gain was abstracted from the medical record and equaled maternal weight at delivery minus self-reported pre-pregnancy weight. Further, gestational weight gain was normalized to gestational age and within pre-pregnancy BMI categories (see Table 3) as defined by the Institute of Medicine.[128] Extended range values for total gestational weight gain and weekly gain in the 2nd and 3rd trimesters were chosen based on available literature indicating risk of APOs within pre-pregnancy BMI categories.[126] Extended range values for the first trimester were not available in the extant literature; therefore, the extended range for the first trimester was determined by expanding the Institute of Medicine recommendation by 0.44 lb/wk in each direction. This corresponds to one standard deviation of weekly weight gain in the Project Viva cohort[127], and is similar to the standard deviation of 1st trimester weekly weight gain in the present sample. Given the lack of support for an extended range in individuals in the lowest BMI category, no extended range was implemented for this group.

Table 3. Gestational Weight Gain

Pre-pregnancy BMI (kg/m^2)	**Institute of Medicine Guidelines**			**Extended Range**		
	Total gain (lb)	**1st Tri gain (lb/wk)**	**2nd and 3rd Tri gain (lb/wk)**	**Total gain (lb)**	**1st Tri gain (lb/wk)**	**2nd and 3rd Tri gain (lb/wk)**
< 18.5	28 – 40	0.09 – 0.34	1.0 – 1.3	*No change*	*No change*	*No change*
18.5 – 24.9	25 – 35	0.09 – 0.34	0.8 – 1.0	22 – 40	-0.35 – 0.78	0.7 – 1.2
25.0 – 29.9	15 – 25	0.09 – 0.34	0.5 – 0.7	4.4 – 35	-0.35 – 0.78	0.1 – 1.06
≥ 30.0	11 – 20	0.09 – 0.34	0.4 – 0.6	0 – 20	-0.35 – 0.78	-0.04 – 0.6

Smoking was assessed via self-report questionnaire in each trimester visit of MoM Health and Pregnancy 24/7. Physical activity was assessed using MoM Health and Pregnancy 24/7's

objective monitoring as described above (see section 3.3.1). Diet quality was measured in each trimester visit of MoM Health and Pregnancy 24/7 using the Diet History Survey and was scored according an adapted Mediterranean Eating Pattern for Americans scoring (see Table 4).[38] Components not measured in Diet History Survey were omitted from scoring, and alcohol was excluded due to the contraindication to alcohol consumption during pregnancy. Sleep duration was assessed using the Pittsburgh Sleep Quality Index self-report questionnaire.[129]

Table 4. Adapted "Mediterranean Eating Pattern for Americans" Diet Quality Scoring

Dietary Category	Score = 1 point	Score = 0 points
Green leafy vegetables	≥ 7 servings/week	< 7 servings/week
Other vegetables	≥ 2 servings/day	< 2 servings/day
Fruit	≥ 1 serving/day	< 1 serving/day
Whole grains	≥ 3 servings/day	< 3 servings/day
Red or processed meat	≤ 3 servings/week	> 3 servings/week
Cheese	≤ 4 servings/week	> 4 servings/week
Beans	≥ 3 servings/week	< 3 servings/week
Sweets and pastries	≤ 4 servings/week	> 4 servings/week

3.3.3 Placental Tissue Analysis

Fetal vascular percentage (FV%) was quantified by performing morphometric analysis on digital images of placenta histology slides. At delivery, MOMI Biobank personnel obtained placenta tissue samples, which were processed for later analysis according to established protocols.[130] Briefly, placenta tissue was collected within 60 minutes of delivery. Approximately 7.5 mm^3 of villous tissue from the central portion of one quadrant of the placenta, free of any grossly visible lesions, was excised and stored in a 10% formalin solution. Following fixation, it was embedded in paraffin wax for storage.

Biobank samples of paraffin-embedded placental tissue were sectioned at 4µm, air dried, and baked at 60^{o} Celsius. Automated staining took place on a Bond III instrument (Leica Biosystems, Buffalo Grove, IL) with prediluted CD34 antibody (clone QBEnd/10, cat# PA0212, Leica Biosystems, Buffalo Grove, IL) and detected using the Bond Polymer Refine Detection Kit (cat# DS9800, Leica Biosystems, Buffalo Grove, IL). Counterstaining with Shandon Instant Hematoxylin was carried out at a 1:10 dilution for 8 minutes (cat# D5773-1L, Sigma-Aldrich, St. Louis, MO). Finally, slides were dehydrated through 70%, 95%, and 100% ETOH and covered with Xylene.

Whole slide scanning and image digitization was performed at 40x magnification on an Aperio AT2 Whole Slide Scanner (Leica Biosystems, Nussloch, Germany). Image analysis was conducted with Aperio ImageScope software using the Positive Pixel Count algorithm (version 9.1, Leica Biosystems, Nussloch, Germany). FV% was quantified as a continuous variable by comparing the number of pixels stained positive for CD34 antigen (highlighting vascular endothelial cells) to the total number of pixels.

Given the novel nature of these analyses two quantification protocols were trialed, and the results of each protocol were compared. In the first protocol, the entire image was analyzed (Whole Slide protocol, Figure 9). This method included all villous structures (stem, intermediate, and terminal villi); if basal plate tissue was present on the slide, it was manually removed from analysis.

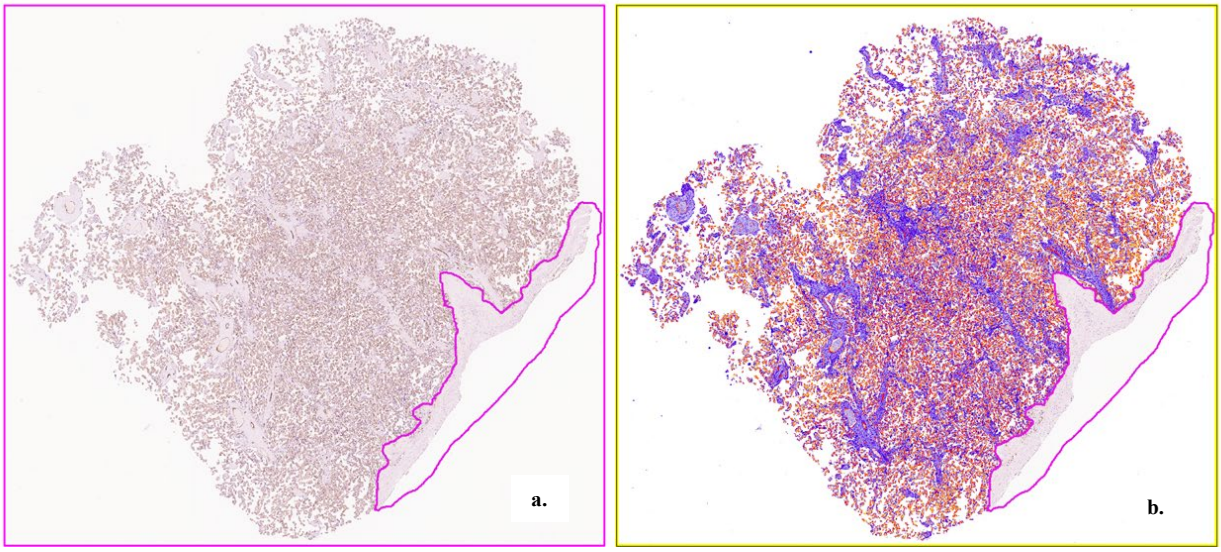
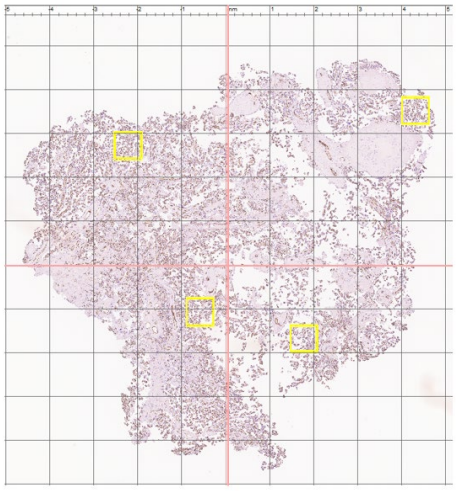


Figure 9. Whole Slide Protocol
9a. Scanned whole slide image of placenta tissue stained for CD34, with a segment of basal plate removed (pink tissue outline). 9b. Marked up image of whole slide analysis, with basal plate removed. Blue mark up indicates areas negative for CD34 antigen, while red and orange mark up indicates areas positive for CD34 antigen.

In the second protocol, the image was divided into four quadrants by centering the image on a grid (Quadrant protocol, Figure 10a). Within each quadrant, a 600µm^2 box of terminal and intermediate villous tissue was randomly selected. The pixel counts of the four boxes were combined for analysis (1200µm^2 of tissue total, Figure 10b-c). Pairwise correlations in the outcome were computed between the protocols.



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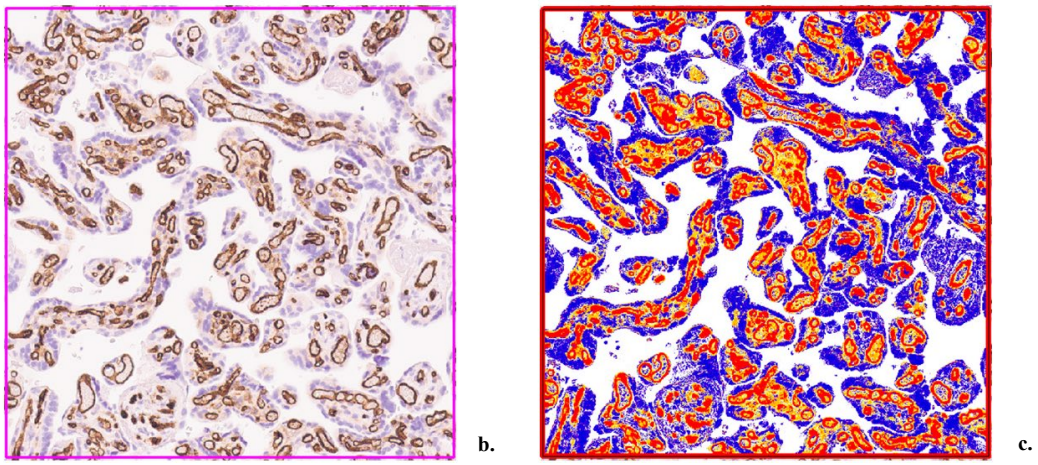


Figure 10. Quadrant Protocol
10a. Whole slide divided into quadrants with selected 600µm^2 boxes in each quadrant. 10b. Zoomed image of one 600µm^2 box encapsulating terminal and intermediate villi. Brown staining indicates vascular endothelial cells positive for CD34 antigen. 10c. Marked up image. Red and orange mark up indicate pixels positive for CD34 antigen, while blue and yellow indicate pixels not positive for CD34 antigen.

The staining and FV% measurement protocols were piloted using placenta tissue from two participants (one each from MoM Health and Pregnancy 24/7). Following protocol piloting, tissue samples for the full cohort were randomly divided into four groups for slide mounting and staining; tissue samples from one participant were included in all four groups as a batch control. All slides were scanned in a single batch upon completion of the staining protocols. Digital analyses for each protocol were repeated in a random subsample to calculate intra-class correlation coefficients for these novel analytic techniques.

3.4 Statistical Analysis

Stata 17 software (StataCorp, College Station, Texas) was used to perform all statistical analyses. Descriptive summaries of sample characteristics and variables for analysis were

performed, and distributions were checked for normality as appropriate. Assumptions of linearity and the normality of residuals were checked for all regression analyses.

Specific Aim 1 was evaluated using two separate approaches. First, mean values of MVPA and sedentary behavior in each of the three trimesters were associated with FV% using linear regression. Next, MVPA and sedentary behavior were averaged across gestation (mean of up to three trimesters) and associated with FV% using linear regression. Mutual adjustment for MVPA and sedentary behavior were considered.

The primary analysis for Specific Aim 2 associated the composite maternal cardiovascular health score averaged across gestation with FV% using linear regression. Exploratory analyses for Specific Aim 2 associated FV% with the following: maternal cardiovascular health scores within each trimester (see Table 5); each component score (averaged across gestation, with trimester-specific scores examined if associations with overall scores were present); and continuous values of blood pressure, sleep, 1-hour glucose screen, pre-pregnancy BMI, and gestational weight gain.

Where statistically significant differences in exposure variables existed between study groups (MoM Health and Pregnancy 24/7), analyses were stratified. Finally, the effect of adjustment for clinically relevant covariates was evaluated, with care to avoid over-adjustment of regression models given the limited number of observations. Covariates considered included pre-pregnancy BMI, smoking status (never versus ever smoker), gestational age, fetal sex, maternal age, maternal race, SARS-CoV-2 infection status, and delivery type. Of note, smoking was excluded as a covariate for composite maternal cardiovascular health scores and the smoking component score as it contributes to those scores. Covariates that showed associations with the FV% outcome at $p \leq 0.2$ were included in regression models.

Statistical significance was set at a two-sided $\alpha=0.05$. Due to our small sample size, we did note associations where $p<0.20$ as approaching statistical significance and we did not adjust our type I error level for multiple comparisons. Given the novel nature of the placenta analysis protocol, preliminary data allowing for *a priori* sample size determination was not available. *Post-hoc* power calculations were conducted to aid in the interpretation of results.

Table 5. Trimester-Specific Maternal Cardiovascular Health Components

First Trimester	Second Trimester	Third Trimester
Blood pressure, weeks 0-13	Blood pressure, weeks 14-26	Blood pressure, weeks 27-delivery
-	1-hour glucose screen	-
Pre-pregnancy BMI	Pre-pregnancy BMI	Pre-pregnancy BMI
Gestational weight gain, weeks 0-13	Gestational weight gain, weeks 0-26	Gestational weight gain, weeks 0-delivery
First trimester diet quality score	Second trimester diet quality score	Third trimester diet quality score
First trimester smoking status	Second trimester smoking status	Third trimester smoking status
First trimester physical activity	Second trimester physical activity	Third trimester physical activity

4.0 Results

The purpose of this study was to examine the relationships between maternal physical activity, sedentary behavior, and cardiovascular health profile during pregnancy with placental villous vascular development (FV%). Results below describe the study participants and the findings of each specific aim.

4.1 Research Participants

A total of 86 participants from MoM Health and Pregnancy 24/7 co-enrolled in the MOMI Biobank and delivered prior to July 1, 2022. Of these, 17 did not provide placenta tissue samples to the Biobank due to delivering off-shift or at a different hospital (4 from MoM Health and 13 from Pregnancy 24/7). Two MoM Health participants were excluded from placenta analysis (one due to twin delivery and one due to neonatal demise secondary to a genetic disorder), and two Pregnancy 24/7 participants were excluded after no exposure data were collected. Finally, one participant enrolled in both MoM Health and Pregnancy 24/7 during two separate pregnancies; data collected in Pregnancy 24/7 was used for this participant. The final sample comprised 64 participants (35 from MoM Health and 29 from Pregnancy 24/7). Participant descriptive data from the final sample are displayed by study in Table 6.

Table 6. Participant Characteristics

	Overall (N=64)	MoM Health (n=35)	Pregnancy 24/7 (n=29)
Maternal age (years)	32.0 ± 4.98	32.1 ± 5.08	31.9 ± 4.94
Non-white race (%)	19%	20%	17%
Black/African American (n)	7	5	2
Native Hawaiian/Pacific Islander (n)	1	0	1
Asian (n)	3	1	2
Other (n)	1	1	0
Hispanic ethnicity (%)	5%	6%	3%
Pre-pregnancy BMI (kg/m^2)	25.8 ± 6.03	26.4 ± 6.43	25.1 ± 5.54
Ever smoker (%)	17%	14%	21%
GA at delivery (weeks)	39.0 ± 1.57	39.2 ± 1.46	38.8 ± 1.68
Female fetal sex (%)	39%	40%	38%
Vaginal delivery (%)	73%	74%	72%
Composite APO (%)	23%	14%	35%
SARS CoV-2 infection (%)	-	-	0%

Continuous variables reported as mean ± SD; categorical variables reported as percentages. GA=gestational age. Composite APOs: at least one of preterm birth, HDP, GDM, or IUGR. SARS CoV-2 infection: % in the Pregnancy 24/7 cohort who experienced infection while pregnant.

Post-hoc power analysis suggested that given the sample size of 64, there was 80% power to detect a small-moderate correlation of 0.33. This correlation corresponds to a β coefficient representing the association between composite maternal cardiovascular health and FV% of approximately 1.5 percentage points (and ranging from 0.4-1.5 percentage points for the individual components of the cardiovascular health score).

4.2 Fetal Vascular Percentage

Mean fetal vascular percentage was significantly greater using the Quadrant protocol compared to the Whole Slide protocol, with a mean difference of five percentage points overall (Table 7). However, no significant differences existed between MoM Health and Pregnancy 24/7 participants for either measure. Furthermore, the values were strongly correlated between the two

protocols (r=0.843, Fig. 11). Finally, intra-rater intraclass correlations were very high for both protocols; for the Whole Slide and Quadrant protocols, respectively, ICCs were 0.999 and 0.975.

Table 7. Fetal Vascular Percentage by protocol

	Whole Slide Protocol	Quadrant Protocol	p-value for protocol	Pearson's Correlation
Overall	26.3 ± 5.13	31.3 ± 5.51	<0.001	0.843
MoM Health	26.7 ± 5.85	32.0 ± 6.07	<0.001	0.851
Pregnancy 24/7	25.7 ± 4.14	30.4 ± 4.72	<0.001	0.826
p-value for study	0.469	0.258	-	-

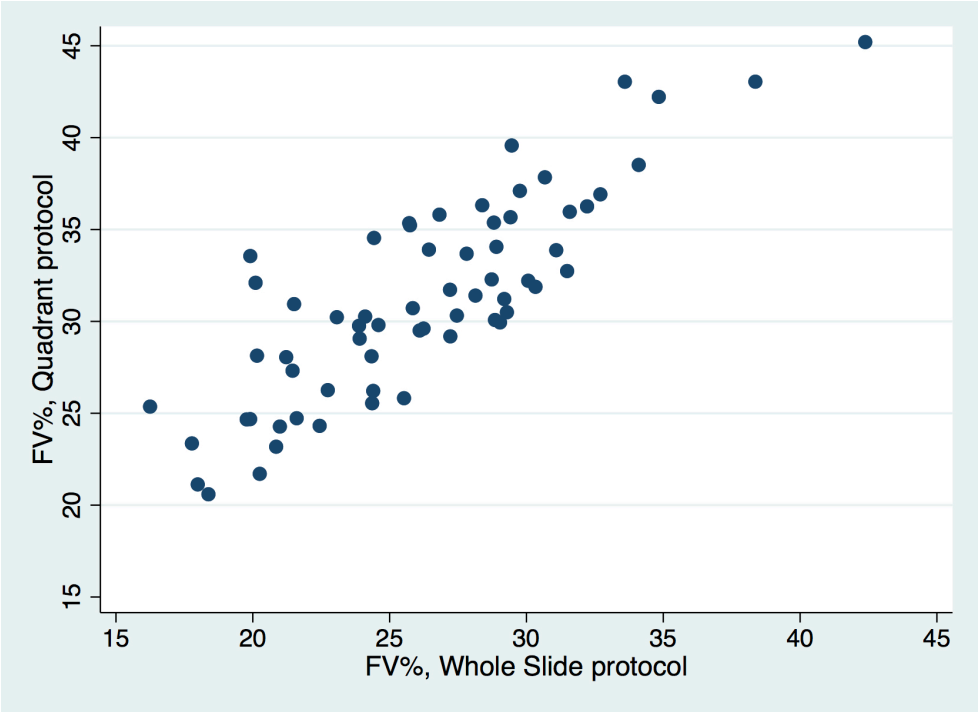


Figure 11. Fetal Vascular Percentage Calculated Using the Whole Slide Protocol Versus the Quadrant Protocol for the Full Study Sample. Pearson's Correlation = 0.843

To choose appropriate covariates for statistical adjustment when addressing the specific aims, univariate associations with both protocols of FV% values were carried out for continuous values of pre-pregnancy BMI, maternal age, and gestational age at delivery, as well as categorical variables of smoking status (ever versus never), fetal sex, maternal race (white versus non-white),

maternal ethnicity (Hispanic versus non-Hispanic), and delivery type (vaginal versus Cesarean section). Only maternal age and smoking status were associated with FV% at $p<0.20$ (data presented in Supplemental Table 1, Appendix A). Therefore, associations for aims 1 and 2 were adjusted for these characteristics only.

4.3 Specific Aim 1: Physical Activity and Sedentary Behavior

4.3.1 Physical activity and sedentary behavior across pregnancy

This specific aim characterized the association between physical activity and sedentary behavior, overall and during each trimester, with FV%. Mean values for physical activity, sedentary behavior, and monitor wear time are presented in Table 8. Significant differences existed between studies at all time points for MVPA, and at the second trimester, third trimester, and overall time points for sedentary behavior. MoM Health participants were typically more active and less sedentary compared to Pregnancy 24/7 participants.

Table 8. Physical Activity and Sedentary Behavior

	Overall (N=64)	MoM Health (n=35)	Pregnancy 24/7 (n=29)	p-value
Overall				
MVPA	166.7 ± 107.41	214.1 ± 104.02	111.1 ± 82.86	***<0.001***
Sedentary behavior	9.82 ± 1.28	9.51 ± 1.11	10.19 ± 1.39	***0.037***
Monitor wear time	910.3 ± 63.1	896.5 ± 49.26	926.8 ± 74.1	0.055
First Trimester				
MVPA	178.1 ± 116.37	212.0 ± 115.27	138.3 ± 106.22	***0.011***
Sedentary behavior	9.89 ± 1.37	9.68 ± 1.28	10.14 ± 1.45	0.181
Monitor wear time	896.4 ± 45.91	891.1 ± 44.25	902.7 ± 47.80	0.323
Second Trimester				
MVPA	176.5 ± 199.99	237.1 ± 114.15	111.7 ± 89.23	***<0.001***
Sedentary behavior	9.55 ± 1.47	9.17 ± 1.21	10.00 ± 1.64	***0.027***
Monitor wear time	910.1 ± 62.03	899.0 ± 57.18	923.2 ± 65.95	0.131
Third Trimester				
MVPA	146.1 ± 119.49	213.1 ± 110.53	76.6 ± 84.33	***<0.001***
Sedentary behavior	9.87 ± 1.47	9.36 ± 1.07	10.46 ± 1.67	***0.004***
Monitor wear time	928.2 ± 135.29	902.1 ± 63.88	957.2 ± 182.24	0.126

Overall values are the average across gestation. Trimester-specific values are those measured in each trimester. Moderate-to-vigorous physical activity (MVPA) and monitor wear time are presented in minutes/week; sedentary behavior is presented in hours/day. Values are presented as mean ± SD

4.3.2 Associations between physical activity and fetal vascular percentage

Physical activity was not significantly associated with FV% using either placental analysis protocol at any time point. A nonsignificant negative association approached statistical significance in the third trimester in the Pregnancy 24/7 cohort using both protocols; this trend was more apparent using the Quadrant protocol, whereby each 10-minute increase in MVPA per week was associated with a 0.16% decrease (95% CI -0.37, 0.05; p=0.130) in FV% when adjusted for monitor wear. This association strengthened with additional covariate adjustment for maternal age and smoking status (β=-0.17; 95% CI -0.38, 0.04; p=0.102). No similar trends were seen in the MoM Health cohort. Full results of physical activity associations using both protocols can be found

in Table 9, and results reported by study can be found in Supplemental Table 2, Appendix A. Finally, further adjustment for sedentary behavior did not change associations.

Table 9. Associations Between MVPA (per 10-minute increase per week) and FV% by Protocol

Physical Activity	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Overall						
Model 1	0.009	-0.12, 0.13	0.890	-0.014	-0.15, 0.11	0.833
Model 2	-0.014	-0.13, 0.11	0.823	-0.029	-0.16, 0.10	0.662
Trimester 1						
Model 1	-0.009	-0.12, 0.10	0.875	-0.042	-0.16, 0.08	0.496
Model 2	-0.038	-0.15, 0.08	0.515	-0.061	-0.18, 0.06	0.323
Trimester 2						
Model 1	0.069	-0.04, 0.18	0.222	0.031	-0.09, 0.15	0.612
Model 2	0.056	-0.06, 0.17	0.332	0.036	-0.09, 0.16	0.561
Trimester 3						
Model 1	-0.007	-0.13, 0.11	0.909	-0.028	-0.15, 0.10	0.651
Model 2	-0.022	-0.14, 0.10	0.707	-0.044	-0.17, 0.08	0.486

Model 1 includes adjustment for monitor wear time. *Model 2* is further adjusted for maternal age and smoking status

4.3.3 Associations between sedentary behavior and fetal vascular percentage

Sedentary behavior was not associated with FV% when examined across gestation or in the first or second trimesters, though a nonsignificant, positive association in the first trimester approached statistical significance after adjustment for maternal age and smoking status (Model 2, Table 10). In the third trimester, sedentary behavior was significantly and negatively associated with the Whole Slide protocol calculation of FV% in the MoM Health cohort (β=-2.43, 95% CI -4.50, -0.36; p=0.023); however, this association was attenuated when using the Quadrant protocol calculation (β=-1.66; 95% CI -3.77, 0.46; p=0.119) and disappeared entirely in both protocols when further adjusted for maternal age and smoking status (p>0.5). For full results stratified by

study, refer to Supplemental Table 3, Appendix A. Additional adjustment for MVPA did not alter associations.

Table 10. Association Between Sedentary Behavior (per 1-hour increase per day) and FV% by Protocol

Sedentary Behavior	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Overall						
Model 1	0.22	-0.81, 1.25	0.671	0.20	-0.90, 1.30	0.721
Model 2	0.33	-0.67, 1.33	0.516	0.22	-0.88, 1.32	0.688
Trimester 1						
Model 1	0.46	-0.50, 1.42	0.337	0.55	-0.47, 1.58	0.285
Model 2	0.73	-0.22, 1.68	0.128	0.69	-0.36, 1.74	0.194
Trimester 2						
Model 1	0.06	-0.92, 1.04	0.904	0.01	-1.04, 1.05	0.990
Model 2	0.08	-0.88, 1.05	0.865	-0.05	-1.10, 0.99	0.917
Trimester 3						
Model 1	-0.46	-1.50, 0.57	0.372	-0.46	-1.52, 0.60	0.386
Model 2	-0.14	-1.17, 0.89	0.789	-0.26	-1.35, 0.82	0.628

Model 1 includes adjustment for monitor wear time. *Model 2* is further adjusted for maternal age and smoking status

4.4 Specific Aim 2: Maternal Cardiovascular Health

4.4.1 Cardiovascular health scores

Composite cardiovascular health scores in the full cohort were, on average, in the "moderate" range on a scale of 0-100, with a mean overall score of 72.6 $\pm$ 10.65 points (Figure 12). Scores decreased significantly across pregnancy, with an average of 65.1 $\pm$ 11.98 points in the third trimester compared to 72.8 $\pm$ 12.71 points in the first trimester ($p<0.01$). Scores in the MoM Health cohort were higher than those in the Pregnancy 24/7 cohort, though these differences did not reach statistical significance until the third trimester.

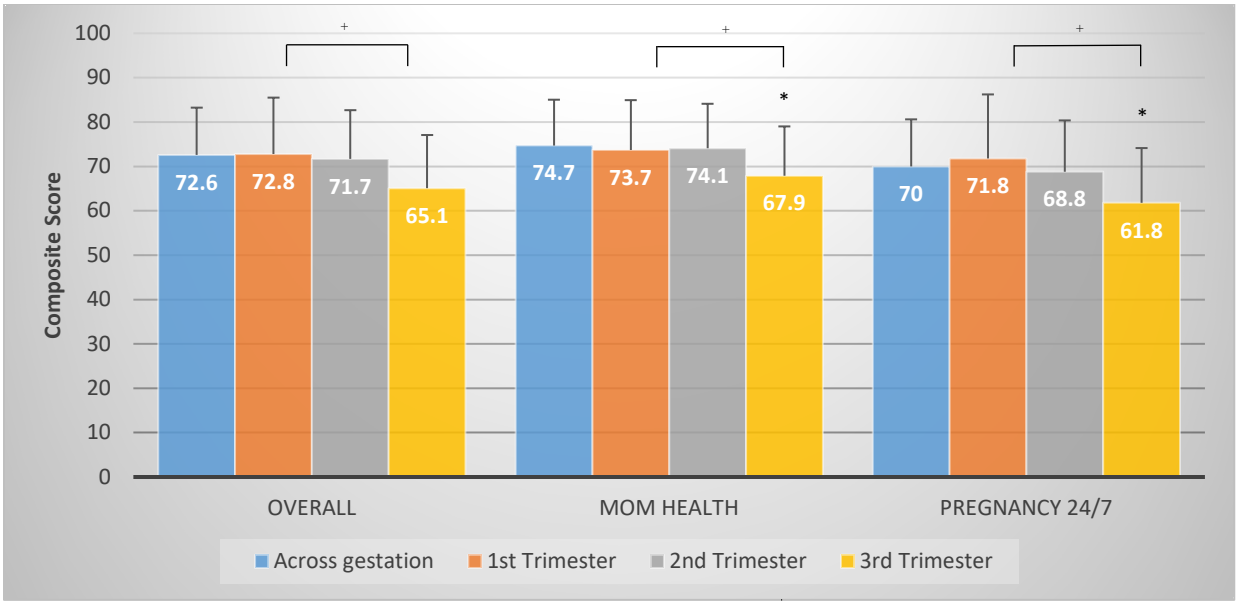


Figure 12. Composite Cardiovascular Health Scores by Study
Data presented as mean ± SD. +Significant difference between 1st and 3rd Trimesters, p<0.01. *Significant difference between MoM Health and Pregnancy 24/7 in 3rd Trimester, p=0.028.

Average scores for the individual components are presented in Figure 13. Scores were "moderate" for pre-pregnancy BMI and "high" in physical activity, smoking, blood pressure, glucose screen, and sleep; diet and gestational weight gain were "poor". Component scores reported by study are presented in Supplemental Figure 1, Appendix B.

Composite scores were excluded from analysis if more than three component scores were missing. Only two participants were excluded from analysis in the third trimester for incomplete cardiovascular health score data. The component that was most frequently missing was physical activity, with data unavailable for six participants in the second trimester and seven participants in the third trimester.

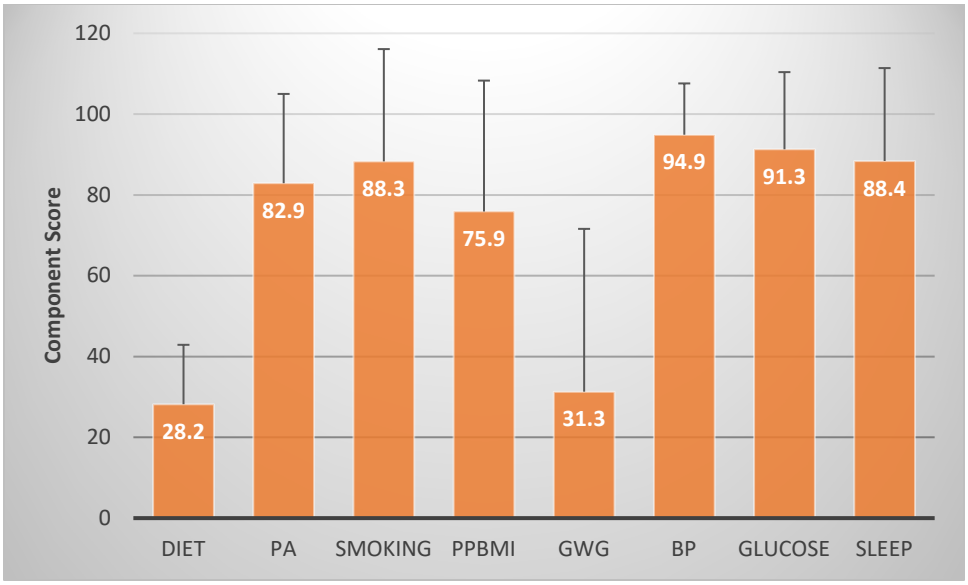


Figure 13. Cardiovascular Health Component Scores, Averaged Across Gestation
Scores range 0-100 points. PA=physical activity; PPBMI=pre-pregnancy body mass index; GWG=gestational weight gain; BP=blood pressure.

4.4.2 Associations between composite scores and fetal vascular percentage

Associations between composite scores and FV%, both across gestation and in each trimester, were nonsignificant but were in the expected direction and approached significance using the Whole Slide protocol (Table 11). With the exception of the first trimester, these trends were attenuated when using the Quadrant protocol. Associations were universally attenuated when adjusting for maternal age. A significant association between the composite cardiovascular health score in the second trimester (per 10-point increase) and FV% using the Whole Slide protocol was observed in the MoM Health cohort (maternal age-adjusted β=1.89; 95% CI 0.12, 3.67; p=0.037). No other associations were found by study (Supplemental Table 4, Appendix A).

Table 11. Association Between Composite Cardiovascular Health Score (per 10-point increase) and FV% by Protocol

Composite CVH score	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Overall						
Model 1	0.82	-0.38, 2.03	0.176	0.60	-0.71, 1.90	0.363
Model 2	0.72	-0.46, 1.90	0.226	0.54	-0.77, 1.85	0.412
Trimester 1						
Model 1	0.75	-0.26, 1.75	0.144	0.89	-0.19, 1.97	0.104
Model 2	0.55	-0.45, 1.56	0.276	0.80	-0.30, 1.91	0.152
Trimester 2						
Model 1	0.77	-0.40, 1.94	0.192	0.60	-0.66, 1.87	0.346
Model 2	0.62	-0.53, 1.77	0.284	0.52	-0.76, 1.80	0.418
Trimester 3						
Model 1	0.91	-0.17, 1.99	0.098	0.34	-0.83, 1.52	0.561
Model 2	0.75	-0.32, 1.82	0.166	0.26	-0.93, 1.45	0.665

Model 1 presents unadjusted values. *Model 2* adjusts for maternal age.

4.4.3 Exploratory analysis of associations between component scores and fetal vascular percentage

No significant associations were found between any of the individual cardiovascular health component scores and FV% with either protocol (Table 12). However, positive associations between the diet score and FV% using the Whole Slide protocol approached statistical significance (p=0.070); this association was strengthened using the Quadrant protocol (p=0.058), though both associations were attenuated when adjusting for maternal age and smoking status. Additionally, a nonsignificant positive association between the overall sleep component score and FV% was seen using the Whole Slide protocol (p=0.113) but was less associated using the Quadrant protocol (p=0.486).

Table 12. Association Between Overall Component Scores (per 10-point increase) and FV% by Protocol

Overall component scores	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Blood pressure						
Model 1	-0.43	-1.45, 0.58	0.398	-0.39	-1.48, 0.71	0.483
Model 2	-0.43	-1.43, 0.57	0.391	-0.46	-1.56, 0.65	0.411
Blood glucose						
Model 1	-0.14	-0.82, 0.53	0.675	-0.14	-0.87, 0.60	0.714
Model 2	-0.26	-0.92, 0.40	0.428	-0.20	-0.94, 0.54	0.588
Pre-pregnancy BMI						
Model 1	0.16	-0.24, 0.56	0.427	0.09	-0.34, 0.53	0.662
Model 2	-0.01	-0.45, 0.43	0.959	-0.09	-0.57, 0.40	0.722
Gestational weight gain						
Model 1	-0.03	-0.35, 0.30	0.874	-0.03	-0.38, 0.32	0.857
Model 2	-0.004	-0.32, 0.31	0.982	-0.03	-0.38, 0.32	0.845
Diet						
Model 1	0.80	-0.07, 1.66	0.070	0.89	-0.03, 1.82	0.058
Model 2	0.62	-0.24, 1.47	0.154	0.76	-0.18, 1.70	0.113
Smoking						
Model 1	0.32	-0.14, 0.78	0.167	0.33	-0.17, 0.83	0.190
*Model 2**	0.23	-0.23, 0.69	0.319	0.29	-0.22, 0.79	0.267
Physical activity						
Model 1	0.24	-0.35, 0.83	0.416	0.17	-0.47, 0.80	0.601
Model 2	0.11	-0.48, 0.70	0.708	0.07	-0.58, 0.71	0.836
Sleep						
Model 1	0.45	-0.11, 1.00	0.113	0.21	-0.39, 0.82	0.486
Model 2	0.46	-0.07, 1.00	0.090	0.20	-0.40, 0.81	0.506

BP=blood pressure; PPBMI=pre-pregnancy body mass index; GWG=gestational weight gain; PA=physical activity. *Model 1* presents unadjusted values. *Model 2* adjusts for maternal age and smoking (*Smoking component score is only adjusted for maternal age).

When interrogated further within trimesters, the relationship between the diet component score and FV% was being driven by a significant positive association in the first trimester. Adjusted for maternal age and smoking status and using the Quadrant protocol calculation of FV%, a 10-point increase in first trimester diet score was associated with a 1.00% increase in FV% (95%

CI 0.18, 1.83; p=0.018). Diet scores in the 2nd and 3rd trimesters were not significantly associated with FV%, but the 2nd trimester score approached significance using the Whole Slide protocol (adjusted β=0.77; 95% CI -0.15, 1.69; p=0.098). Results reported by trimester can be found in Supplemental Table 5, Appendix A.

4.4.4 Exploratory analysis of associations between continuous values of cardiovascular health components and fetal vascular percentage

Associations between continuous values of systolic blood pressure, diastolic blood pressure, gestational weight gain, and sleep with FV% were not statistically significant across gestation or within any trimester. Associations between first trimester diastolic blood pressure and FV% approached significance using both protocols (Quadrant protocol adjusted β=0.15; 95% CI -0.028, 0.326; p=0.098).

Continuous values of pre-pregnancy BMI were not associated with FV%. Associations between continuous values of the 1-hour glucose screen and FV% also approached significance using both protocols (Quadrant protocol adjusted β=0.038; 95% CI -0.009, 0.086; p=0.111). Full results for associations between continuous component values can be found in Supplemental Table 6, Appendix A.

5.0 Discussion

5.1 Summary

The purpose of this study was to examine the association between maternal physical activity, sedentary behavior, and cardiovascular health during pregnancy with the development of the fetal vasculature in the intermediate and terminal villi (FV%). It is well-known that abnormalities in placental development are related to APOs, but the mechanistic antecedents of this relationship are not yet fully understood. Furthermore, research indicates that maternal behaviors such as physical activity and sedentary behavior, as well as other behaviors and clinical characteristics that comprise overall maternal cardiovascular health, are also linked with the incidence of APOs. Therefore, this study set out to investigate whether these maternal characteristics were directly associated with FV% as a measure of placental health.

Overall, significant associations were not found between the exposures and FV% in this combined observational cohort. However, associations approached statistical significance in a number of the relationships that were examined. In particular, nonsignificant associations in the hypothesized direction were found for the composite maternal cardiovascular health scores and a significant association with the diet score was found in the first trimester.

Finally, this project provides useful preliminary data regarding a novel analytic technique for continuously quantifying vascular development in the placenta. Two protocols were utilized and compared against the exposures of interest. While strongly correlated, the two methods did classify FV% differently in potentially important ways that may provide guidance for future

research. Below, we will first describe knowledge gained regarding the quantification of FV%, followed by contextualization and future directions for the research findings in each Specific Aim.

5.2 Quantitative Analysis of Placenta Tissue

Intra-rater intraclass correlations (ICC) were very high for both protocols, indicating acceptable test-retest reliability. The ICC for the Whole Slide protocol was 0.999; differences for this protocol were attributable to variations in the manual procedure of removing basal plate tissue from analysis. Similarly, the ICC for the Quadrant protocol was 0.975. This represented very high concordance between tests, and captured slight variation in FV% when different random sections of tissue were selected within each quadrant. Overall, quantitative analysis using either technique is highly reliable.

Yet, significant differences existed between the two protocols used to quantify FV% in this sample. FV% was, on average, 5 percentage points higher when using the Quadrant protocol compared to when using the Whole Slide protocol. Given the anatomical differences between stem villi and intermediate/terminal villi, this difference was expected. Whole Slide analysis included all villous structures; as such, the stromal tissue that predominates in stem villi was included in the denominator of the FV% calculation. Vessel structures occupy a smaller proportion of stem villi compared to intermediate and terminal villi, thus contributing fewer pixels to the numerator per similar surface area.[49]

While FV% results were higher when using the Quadrant as compared to the Whole Slide calculation, the magnitude of the increase varied across participants. The range of the difference between the two calculations was 0.29-13.6 percentage points. Still, the Pearson's correlation was

strongly positive at r=0.843. This likely indicates that compared to the Quadrant protocol, the Whole Slide protocol may have misclassified the true FV% in the intermediate and terminal villi for a subset of participants. The images in Figure 14 depict two samples that varied widely in FV% using the Whole Slide protocol (Fig. 14a: FV%=19.9%; Fig. 14b: FV%=30.3%) but were very similar using the Quadrant protocol (Fig. 14a: FV%=33.6%; Fig. 14b: FV%=31.9%). As placenta sampling was undertaken using established protocols, variation in the proportion of tissue space occupied by stem villi may have been random. However, the possibility that placenta samples with varying proportions of stem villi were obtained for reasons relating to important participant characteristics or unmeasured confounding cannot be ruled out. Recent imaging using 3-dimensional optical coherence tomography found differences in the number, length, and diameter of intermediate and terminal villi in placentas from uncomplicated pregnancies and those complicated by gestational hypertension, gestational diabetes, and fetal growth restriction.[131] It is possible that observed differences in villous architecture in the present study may be related to similar health conditions. Future research can elucidate these distinctions by examining the relationships between the two calculations and outcomes such as APOs and markers of infant health such as birthweight or ponderal index.

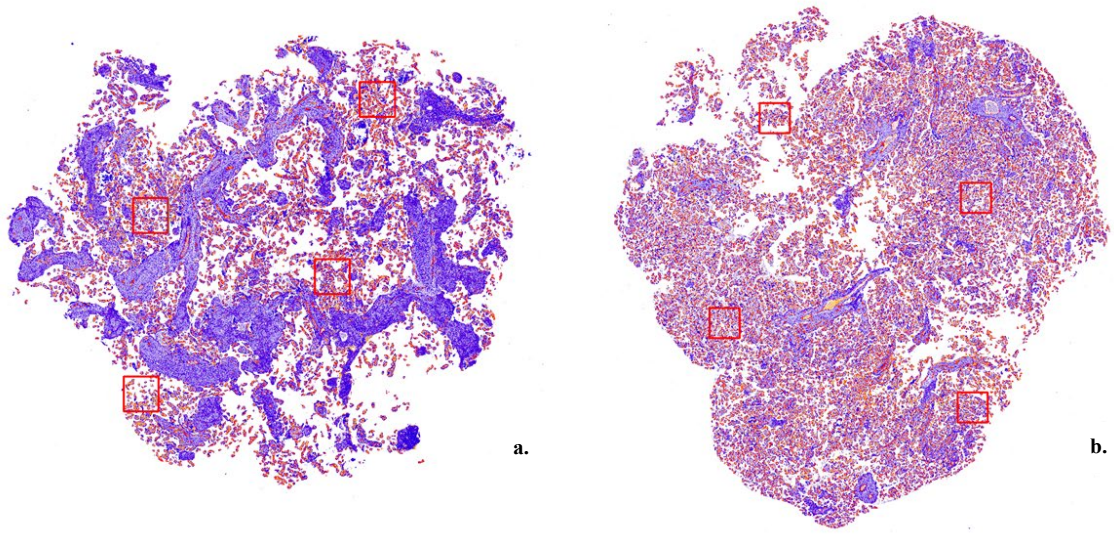


Figure 14. Whole Slide Images After Mark-Up
14a. Whole slide image is predominately stem villi with low vascularization (dark blue areas), but with well-vascularized intermediate and terminal villi (red boxes). 14b. Whole slide image contains few stem villi; intermediate and terminal villi are well-vascularized.

5.3 Physical Activity and Sedentary Behavior

5.3.1 Physical Activity

The hypothesis that higher physical activity levels would be associated with greater FV% was not supported at any time point. Previous literature has found morphometric differences in placental vascularization between participants who participated in higher intensity structured exercise such as running during pregnancy (higher vascular volume) and those who did not participate in regular MVPA during pregnancy (lower vascular volume).[13,14] Further, recent research has found significant differences in expression of mRNA and proteins related to angiogenesis between those who participated in prescribed aerobic exercise in the latter half of

pregnancy and those who remained sedentary; those who participated in exercise were found to have greater quantities of angiogenic biomarkers in the placenta than those who did not.[109] The lack of support for the hypothesized association in the current study may be related to the observational study design or the methodology used to quantify physical activity. Specifically, MVPA was quantified as the accumulation of all minutes above the threshold of 3.0 metabolic equivalents (METs), and was not required to be undertaken as structured exercise or with any requirements for sustained intensity across a given duration (i.e., bouts). This definition of physical activity, though consistent with current guidelines for the maintenance of health in general and pregnant populations[81,82,86], may not have allowed for enough stimulus to demonstrate a relationship with FV%. Future research that utilizes a randomized clinical trial of structured exercise, or observational data that associates bouted physical activity at a variety of intensities with FV%, could help to determine whether the null results of this study are due to misclassification of the exposure or, rather, that FV% measured in this way is truly not related to physical activity.

Unexpectedly, the association between third trimester MVPA and FV% approached statistical significance in the Pregnancy 24/7 cohort, whereby greater physical activity per week was related to lower FV%. However, this may have been influenced by an outlier with very high levels of MVPA (see Supplemental Figure 2 in Appendix B). While few participants accumulated greater than 300 min/week of MVPA, it is of note that the corresponding FV% values were typically low in these participants. Further research should investigate the association between very high weekly physical activity and placental vascular development, as it is possible the relationship between MVPA and FV% is non-linear.

5.3.2 Sedentary Behavior

It was hypothesized that higher sedentary behavior during pregnancy would be associated with lower FV%. The present data do not support this hypothesis, but do offer some interesting insights for consideration in future research. Following adjustment for maternal age and smoking status, first trimester associations approached statistical significance in both protocols. However, these associations were in the opposite direction than was hypothesized, whereby higher sedentary behavior in the first trimester was associated with higher FV%. While the reasons for this finding are unclear, prior research indicates that higher sedentary behavior in the first trimester could be related to pregnancy-associated symptoms of nausea and fatigue[132]; one speculation is that these physical symptoms during early pregnancy may be an evolutionary encouragement to rest and could assist in early placental angiogenesis. Additional research is required to elucidate this relationship. Very limited research has examined associations between sedentary time and placental outcomes, though it appears high levels of sedentary behavior during pregnancy may be linked to APOs.[35,112] Initial steps for further research may include repeating the present analyses in a fully-powered cohort, as well as examining the relationship between sedentary behavior across pregnancy and markers of angiogenesis, inflammation, and oxidative stress in placental tissue and maternal blood.

5.4 Maternal Cardiovascular Health

5.4.1 Maternal cardiovascular health scores across pregnancy

The participants in this study displayed moderate levels of cardiovascular health as measured by an adapted "Life's Essential 8" score.[38] Mean cardiovascular health score decreased across trimesters, indicating an increased cardiovascular burden as pregnancy progressed. This finding is consistent with prior evidence indicating cardiovascular health scores decrease across pregnancy.[114] Furthermore, differences existed by study subsample; Pregnancy 24/7 participants had significantly lower cardiovascular health scores than MoM Health participants in the third trimester. Contrary to the MoM Health cohort that had no exposure to the SARS-CoV-2 virus and related societal implications, Pregnancy 24/7 participants were recruited exclusively during the pandemic; this may have impacted cardiovascular health scores in a number of ways. Indeed, APOs were twice as high in the Pregnancy 24/7 participants as compared to the MoM Health participants in spite of no participants having evidence of COVID infection during pregnancy. It is possible a period effect occurred where that the experience of living during the pandemic may have led to worse cardiovascular health and subsequent adverse outcomes, which have reportedly increased during the pandemic.[133]

Normative values for cardiovascular health scores during pregnancy using the updated American Heart Association composite score[38] are not yet available, but a study using a prior iteration of the cardiovascular health metric found that pregnant individuals displayed moderate-level scores on average.[114] Results of the present study support these findings, with mean values falling within the "moderate" range of 50-79 points.[38] Moreover, patterns within each component

in this study were similar to previously published literature; diet scores were the most commonly poor, while blood pressure and glucose were typically in the ideal range.[114]

Sleep was added as a new component with the recent update from the Simple 7 to the Essential 8 framework of ideal cardiovascular health. Three-quarters of the present sample scored 90 or 100 points (ideal) on sleep averaged across gestation, while 9 participants scored 70 points (moderate) and 6 scored less than 50 points (poor). This indicates that the majority of the present sample was achieving ideal sleep duration during pregnancy according to population-level recommendations. As no pregnancy-specific sleep recommendations have been published, it is not clear whether the observed sleep durations were appropriate for ideal pregnancy health.

5.4.2 Composite cardiovascular health score and FV%

While no significant associations were detected between maternal cardiovascular health score and FV%, either overall or within any single trimester, all of the associations were in the hypothesized direction. Specifically, as the composite score increased, so too did FV%. In all but the first trimester, the observed associations were stronger using the Whole Slide protocol, which was impacted by the presence or absence of a high area of stem villi in the sampled tissue. While it is still unclear whether this presence or absence was random or related to pregnancy health, it is possible that participants with worse (lower) cardiovascular health composite scores had placentas with a greater proportion of stem villi compared to intermediate and terminal villi. If true, this could suggest that poorer cardiovascular health during pregnancy was related to impaired development of the intermediate and terminal villous structures that support nutrient transfer between maternal and fetal circulation. Further study of this hypothesized mechanism is warranted.

While associations between cardiovascular health composite score and FV% were attenuated using the Quadrant protocol in the second and third trimesters compared to the Whole Slide protocol, associations were stronger in the first trimester using the Quadrant protocol. This suggests that the first trimester may be an important time to target improved cardiovascular health so as to provide optimal conditions for the developing placental vascular bed. However, it is important to remember that the observed associations were not statistically significant and must be interpreted with caution.

Lastly and importantly, *post-hoc* power analysis indicated this study was powered to detect a 1.5 percentage-point change in FV% for each 10-point change in maternal cardiovascular health score. The largest magnitude of change observed in FV% was 0.89 percentage points per 10-point change in cardiovascular health score in the unadjusted model of first trimester using the Quadrant protocol. Therefore, it is probable this study was underpowered to detect significant associations between cardiovascular health scores and FV% and the likelihood of a type II error is high. Future studies that are fully-powered to detect statistically significant associations based on these preliminary findings are warranted.

5.4.3 Relationship between individual cardiovascular health components and FV%

While no significant associations between individual component scores (using the scoring in the Life's Essential 8 framework) and FV% were detected in the analyses across gestation, a 10-point increase in diet score in the first trimester was significantly associated with a 1 percentage-point increase in FV%. Diet was the component with the lowest mean score in all three trimesters, and only 3 participants achieved an "ideal" score of 80 or higher in the first trimester. The adapted Mediterranean Eating Pattern for Americans used in this study emphasized the

consumption of green leafy vegetables, fruits, legumes, and whole grains while limiting red and processed meats, cheese, and sweets and pastries. Prior research indicates that pregnant participants in North America frequently fail to meet the recommended dietary standards, but that better diet quality during pregnancy is linked to more favorable maternal and child outcomes.[134–136] The results of the present study support these findings, indicating diet may be a key area for intervention in early pregnancy.

Continuous values of cardiovascular health components, including systolic and diastolic blood pressure, gestational weight gain, sleep, pre-pregnancy BMI, and one-hour glucose screening results, were not significantly related to changes in FV%. First trimester diastolic blood pressure and one-hour glucose screen results both approached significance. In both cases, the association was in the positive direction meaning that higher first trimester diastolic blood pressure and higher blood glucose levels were associated with higher FV%. These are in the opposite direction as hypothesized. Additional research is required to confirm the presence of these unexpected associations and to understand the nature of these unexpected relationships between these continuous values and vascular development in the placenta.

5.5 Strengths and Limitations

This study has a number of strengths. Objective monitoring of physical activity and sedentary behavior provided more accurate data than self-report questionnaires. In addition, since the placenta tissue samples came from participants who co-enrolled in the MOMI Biobank prior to delivery, this sample was not impacted by the selection bias that could occur with reliance on placental pathology reports that are indicated in the event of pregnancy complications.

Furthermore, this study benefitted from a prospective design for collection of exposure data; this allowed for analyses across gestation as well as within each trimester.

The primary limitation of this study was the inadequate sample size that made findings vulnerable to type II error. This was further complicated by significant differences between the MoM Health and Pregnancy 24/7 cohorts on some exposure variables; this increased variability, but also could have confounded results in unpredictable ways. This encouraged additional model stratification, further limiting sample size. The reasons for these differences are not clear; it is possible the experience of being pregnant during the pandemic impacted behavior and health. It is also possible the option for fully virtual study assessments in Pregnancy 24/7 yielded a sample of individuals who were less active overall than those who consented to participate in MoM Health, where in-person assessments were required. Finally, while both studies endeavored to recruit individuals from racially diverse backgrounds, the final sample was predominantly white. This limits the generalizability of these findings to non-white populations.

5.6 Future Directions

This research highlights a number of future directions for inquiry. First, repeating these analyses with an adequate sample to achieve statistical power is necessary to elucidate the true nature of the relationships between physical activity, sedentary behavior, and maternal cardiovascular health exposures during pregnancy and the outcome of FV%. Furthermore, examining the impact of physical activity and/or sedentary behavior interventions, where the exposures are randomized and prescribed, will bolster understanding of the relationship between intensity and/or dose of activity and FV%. Interventions to improve cardiovascular health profile

in early pregnancy, particularly with regards to diet, may be promising avenues for future study. Additionally, while this research successfully captured exposure data in the first trimester, understanding how the pre-conception maternal cardiovascular profile impacts placentation and vascular growth is required to determine the relative impacts of pre-conception health and systemic changes to the maternal cardiovascular system due to pregnancy on placental health. Also, no data exist describing how fetal vascular development in the placenta relates to long-term maternal cardiovascular health. Examining whether FV% is an informative predictor of risk of development of cardiovascular disease may guide future clinical practice. Finally, further interrogating the utility of the two placenta tissue analysis protocols to provide information about healthy pregnancies and those complicated by various adverse outcomes is necessary to determine clinically relevant effect sizes and applications.

5.7 Conclusions

The results of this study did not support the hypothesized associations between higher maternal physical activity, lower sedentary behavior, and greater cardiovascular health during pregnancy and with higher levels of vascular development in the placenta. However, the low sample size may have impacted the power to detect associations with FV%. In particular, the direction of the findings in Specific Aim 2 relating to maternal cardiovascular health scores across pregnancy indicated higher cardiovascular health scores may be important for placental development as measured by FV%. Additional study is required to determine the true nature of these associations.

In addition, this study provided important information about a novel analytic technique for quantifying fetal vasculature within the placenta. Using immunohistochemistry and a computer-assisted pixel counting algorithm, a continuous variable describing the percentage of villous space occupied by fetal vasculature was created. Significant differences existed between the two analytic protocols employed in this study. Analyzing the whole slide (after exclusion of basal plate tissue) yielded lower FV% values than analyzing sections of tissue that were solely comprised of intermediate and terminal villi. Future research should investigate the maternal and fetal health outcomes that are associated with these analytic methods so as to inform its utility in research and clinical settings.

Appendix A Supplemental Tables

Supplemental Table 1. Univariate Associations Between Covariates and FV%

	Whole Slide Protocol			**Quadrant Protocol**		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Pre-pregnancy BMI	-0.06	-0.27, 0.16	0.593	-0.02	-0.26, 0.21	0.837
Smoking	*-2.84*	*-6.19, 0.50*	*0.095*	*-2.85*	*-6.46, 0.76*	*0.120*
Fetal sex	0.89	-1.75, 3.53	0.502	-1.21	-4.04, 1.62	0.396
Maternal age	***0.27***	***0.02, 0.53***	***0.034***	0.15	-0.13, 0.43	0.279
Maternal race	1.19	-2.11, 4.48	0.474	1.84	-1.69, 5.37	0.301
Maternal ethnicity	0.71	-5.40, 6.82	0.818	-0.38	-6.95, 6.19	0.908
Delivery type	0.63	-2.29, 3.55	0.669	-0.02	-3.17, 3.12	0.989
Gestational age at delivery	-0.24	-1.07, 0.58	0.558	-0.18	-1.07, 0.71	0.689

Supplemental Table 2. Association Between MVPA (per 10-minute increase per week) and FV%, by Study and Protocol

Physical Activity	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
MoM Health Cohort						
Overall						
Model 1	0.010	-0.20, 0.22	0.923	-0.058	-0.27, 0.15	0.579
Model 2	-0.015	-0.20, 0.17	0.873	-0.076	-0.28, 0.13	0.455
Trimester 1						
Model 1	-0.010	-0.20, 0.18	0.916	-0.072	-0.26, 0.12	0.447
Model 2	-0.023	-0.19, 0.14	0.780	-0.081	-0.27, 0.10	0.373
Trimester 2						
Model 1	0.095	-0.10, 0.29	0.323	-0.005	-0.20, 0.19	0.961
Model 2	0.062	-0.13, 0.25	0.510	-0.018	-0.22, 0.19	0.862
Trimester 3						
Model 1	-0.004	-0.23, 0.22	0.971	-0.079	-0.31, 0.15	0.482
Model 2	-0.062	-0.27, 0.14	0.536	-0.126	-0.35, 0.09	0.247
Pregnancy 24/7 Cohort						
Overall						
Model 1	-0.064	-0.26, 0.13	0.512	-0.087	-0.31, 0.14	0.430
Model 2	-0.083	-0.30, 0.13	0.433	-0.11	-0.34, 0.12	0.348
Trimester 1						
Model 1	-0.041	-0.20, 0.12	0.594	-0.065	-0.24, 0.11	0.456
Model 2	-0.053	-0.23, 0.12	0.529	-0.072	-0.26, 0.12	0.442
Trimester 2						
Model 1	-0.004	-0.19, 0.19	0.964	-0.031	-0.25, 0.18	0.767
Model 2	0.002	-0.20, 0.20	0.984	-0.012	-0.22, 0.20	0.908
Trimester 3						
Model 1	-0.141	-0.33, 0.04	0.130	-0.159	-0.37, 0.05	0.130
Model 2	-0.146	-0.34, 0.05	0.136	-0.171	-0.38, 0.04	0.102

Model 1 includes adjustment for monitor wear time. *Model 2* is further adjusted for maternal age and smoking status

Supplemental Table 3. Association Between Sedentary Behavior (per 1-hour increase per day) and FV% by Study and Protocol

Sedentary Behavior	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
MoM Health Cohort						
Overall						
Model 1	-0.17	-2.05, 1.72	0.856	0.53	-1.41, 2.47	0.581
Model 2	0.42	-1.41, 2.24	0.645	1.04	-0.96, 3.05	0.295
Trimester 1						
Model 1	0.32	-1.40, 2.04	0.704	0.98	-0.75, 2.71	0.257
Model 2	0.89	-0.72, 2.51	0.268	1.49	-0.27, 3.24	0.093
Trimester 2						
Model 1	0.30	-1.52, 2.12	0.740	0.90	-0.92, 2.73	0.321
Model 2	0.19	-1.58, 1.96	0.827	0.78	-1.12, 2.68	0.407
Trimester 3						
Model 1	***-2.43***	***-4.50, -0.36***	***0.023***	-1.65	-3.77, 0.46	0.119
Model 2	-0.71	-3.03, 1.61	0.534	-0.45	-2.99, 2.09	0.717
Pregnancy 24/7 Cohort						
Overall						
Model 1	0.77	-0.39, 1.93	0.185	0.34	-1.00, 1.68	0.609
Model 2	0.80	-0.40, 2.00	0.182	0.41	-0.92, 1.74	0.535
Trimester 1						
Model 1	0.83	-0.30, 1.95	0.143	0.55	-0.77, 1.87	0.400
Model 2	0.85	-0.33, 2.03	0.151	0.51	-0.84, 1.85	0.444
Trimester 2						
Model 1	0.18	-0.94, 1.31	0.738	-0.26	-1.53, 1.02	0.683
Model 2	0.20	-0.95, 1.35	0.718	-0.23	-1.45, 0.99	0.702
Trimester 3						
Model 1	0.79	-0.31, 1.88	0.150	0.51	-0.75, 1.78	0.410
Model 2	0.81	-0.33, 1.94	0.156	0.55	-0.71, 1.80	0.377

Model 1 includes adjustment for monitor wear time. *Model 2* is further adjusted for maternal age and smoking status

Supplemental Table 4. Association Between Composite Cardiovascular Health Score (per 10-point increase) and FV% by Study and Protocol

Composite CVH score	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
MoM Health Cohort						
Overall						
Model 1	1.22	-0.72, 3.18	0.211	0.20	-1.87, 2.28	0.843
Model 2	1.38	-0.37, 3.15	0.120	0.32	-1.67, 2.31	0.743
Trimester 1						
Model 1	1.26	-0.53, 3.05	0.161	1.21	-0.65, 3.08	0.194
Model 2	1.36	-0.25, 2.97	0.096	1.29	-0.49, 3.07	0.149
Trimester 2						
Model 1	1.60	-0.38, 3.59	0.110	0.57	-1.56, 2.71	0.588
Model 2	***1.89***	***0.12, 3.67***	***0.037***	0.79	-1.26, 2.84	0.437
Trimester 3						
Model 1	1.46	-0.43, 3.34	0.126	0.29	-1.72, 2.31	0.770
Model 2	1.51	-0.21, 3.22	0.082	0.33	-1.61, 2.28	0.731
Pregnancy 24/7 Cohort						
Overall						
Model 1	0.23	-1.31, 1.77	0.762	0.74	-0.99, 2.48	0.385
Model 2	0.28	-1.34, 1.90	0.723	1.01	-0.77, 2.79	0.255
Trimester 1						
Model 1	0.33	-0.80, 1.45	0.557	0.58	-0.69, 1.85	0.359
Model 2	0.46	-0.82, 1.73	0.467	1.02	-0.37, 2.41	0.142
Trimester 2						
Model 1	-0.11	-1.52, 1.32	0.880	0.35	-1.26, 1.95	0.663
Model 2	-0.07	-1.63, 1.49	0.930	0.72	-1.01, 2.45	0.402
Trimester 3						
Model 1	0.35	-0.96, 1.67	0.586	0.11	-1.40, 1.62	0.886
Model 2	0.45	-0.98, 1.89	0.522	0.39	-1.23, 2.01	0.622

Model 1 presents unadjusted results. *Model 2* is adjusted for maternal age

Supplemental Table 5. Association Between Cardiovascular Health Component Scores Averaged Across Gestation (per 10-point increase) and FV% by Trimester and Protocol

Overall component scores	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Blood pressure						
Trimester 1						
Model 1	-0.21	-0.79, 0.37	0.475	-0.18	-0.80, 0.45	0.578
Model 2	-0.22	-0.79, 0.36	0.458	-0.23	-0.87, 0.41	0.480
Trimester 2						
Model 1	-0.43	-1.39, 0.53	0.373	-0.48	-1.51, 0.55	0.351
Model 2	-0.49	-1.45, 0.46	0.305	-0.63	-1.68, 0.42	0.237
Trimester 3						
Model 1	-0.33	-1.12, 0.46	0.404	-0.26	-1.11, 0.60	0.550
Model 2	-0.46	-1.27, 0.36	0.265	-0.45	-1.35, 0.55	0.321
Gestational weight gain						
Trimester 1						
Model 1	0.11	-0.27, 0.49	0.567	0.31	-0.09, 0.71	0.126
Model 2	0.05	-0.32, 0.42	0.782	0.26	-0.14, 0.66	0.196
Trimester 2						
Model 1	-0.04	-0.47, 0.39	0.838	0.09	-0.37, 0.55	0.707
Model 2	-0.03	-0.45, 0.38	0.870	0.09	-0.37, 0.55	0.699
Trimester 3						
Model 1	0.03	-0.50, 0.55	0.920	-0.23	-0.79, 0.33	0.416
Model 2	0.02	-0.49, 0.52	0.949	-0.23	-0.79, 0.33	0.416
Diet						
Trimester 1						
Model 1	0.64	-0.17, 1.44	0.120	***1.05***	***0.22, 1.88***	***0.014***
Model 2	0.59	-0.19, 1.37	0.135	***1.00***	***0.18, 1.83***	***0.018***
Trimester 2						
Model 1	0.91	-0.01, 1.83	0.053	0.64	-0.35, 1.63	0.203
Model 2	0.77	-0.15, 1.69	0.098	0.47	-0.55, 1.49	0.361
Trimester 3						
Model 1	0.58	-0.16, 1.32	0.124	0.39	-0.42, 1.19	0.339
Model 2	0.39	-0.36, 1.13	0.301	0.26	-0.57, 1.08	0.536
Smoking						
Trimester 1						
Model 1	0.34	-0.15, 0.84	0.171	0.36	-0.17, 0.89	0.180
*Model 2**	0.24	-0.26, 0.74	0.340	0.31	-0.24, 0.86	0.260
Trimester 2						
Model 1	0.34	-0.13, 0.80	0.151	0.34	-0.17, 0.84	0.186
*Model 2**	0.23	-0.23, 0.69	0.312	0.29	-0.23, 0.80	0.270
Trimester 3						
Model 1	0.38	-0.16, 0.93	0.163	0.46	-0.11, 1.04	0.114
*Model 2**	0.30	-0.24, 0.84	0.274	0.43	-0.16, 1.02	0.152

Physical activity						
Trimester 1						
Model 1	0.16	-0.38, 0.71	0.549	0.14	-0.44, 0.73	0.623
Model 2	0.08	-0.46, 0.61	0.777	0.09	-0.50, 0.67	0.771
Trimester 2						
Model 1	0.38	-0.17, 0.93	0.173	0.40	-0.18, 0.98	0.171
Model 2	0.30	-0.25, 0.86	0.279	0.39	-0.19, 0.97	0.187
Trimester 3						
Model 1	0.18	-0.28, 0.65	0.430	0.13	-0.37, 0.69	0.594
Model 2	0.12	-0.35, 0.59	0.606	0.08	-0.44, 0.59	0.771
Sleep						
Trimester 1						
Model 1	0.44	-0.13, 1.01	0.127	0.29	-0.33, 0.91	0.348
Model 2	0.22	-0.37, 0.82	0.455	0.15	-0.51, 0.81	0.641
Trimester 2						
Model 1	0.38	-0.20, 0.95	0.196	0.14	-0.48, 0.77	0.647
Model 2	0.37	-0.19, 0.92	0.196	0.12	-0.50, 0.74	0.702
Trimester 3						
Model 1	0.34	-0.18, 0.86	0.196	-0.13	-0.69, 0.43	0.646
Model 2	0.29	-0.22, 0.80	0.265	-0.19	-0.76, 0.37	0.499

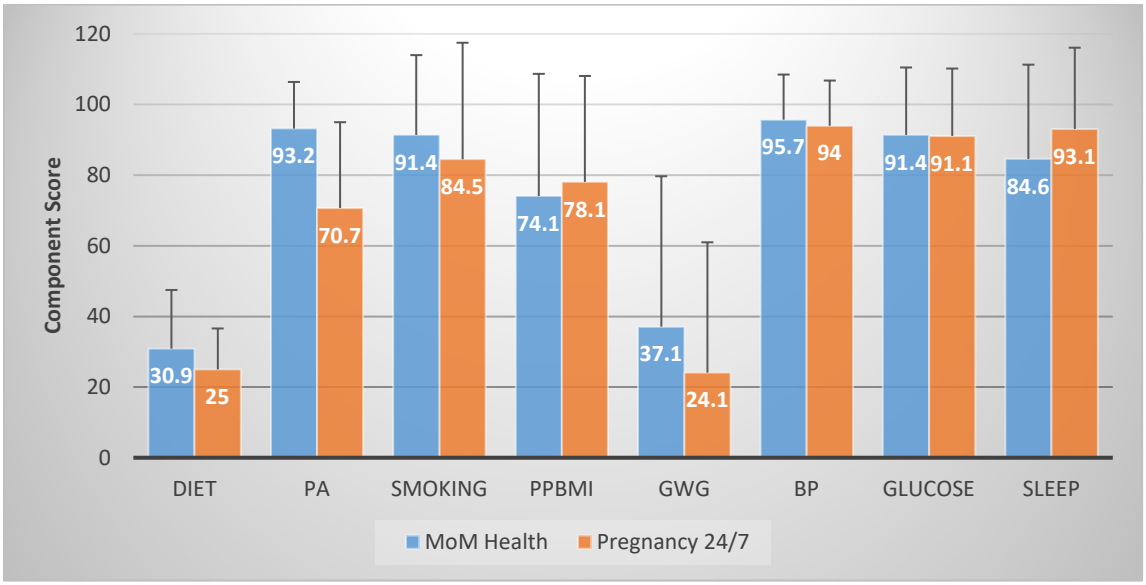
Model 1 presents unadjusted results. *Model 2* is adjusted for maternal age and smoking status. *Smoking component score is only adjusted for maternal age

Supplemental Table 6. Association Between Continuous Values of Cardiovascular Health Components and FV% by Protocol

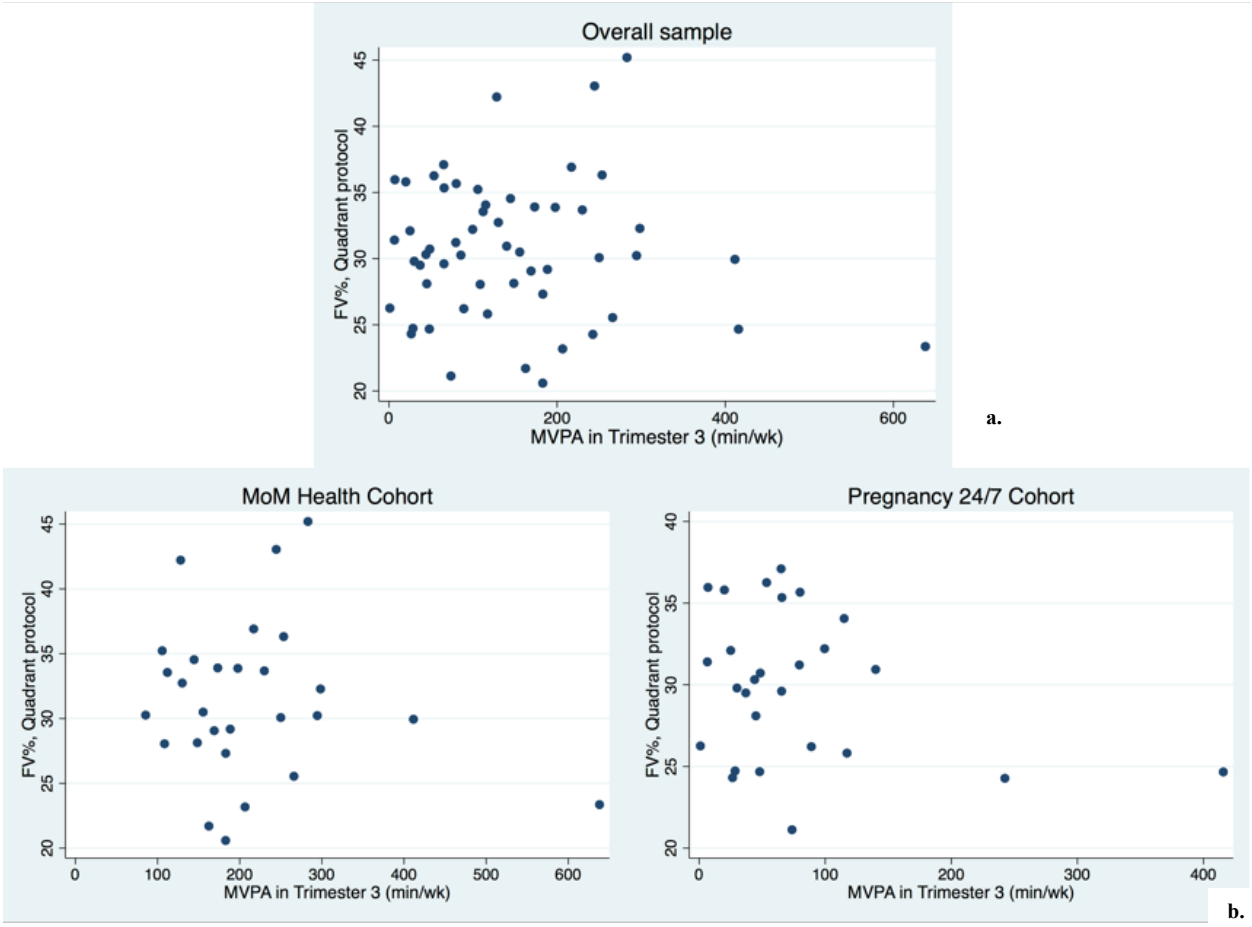
	Whole Slide Protocol			Quadrant Protocol		
	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Systolic BP						
Model 1	-0.03	-0.19, 0.14	0.736	-0.05	-0.23, 0.13	0.569
Model 2	0.02	-0.16, 0.19	0.860	-0.01	-0.20, 0.18	0.949
Diastolic BP						
Model 1	0.07	-0.16, 0.30	0.553	0.09	-0.16, 0.34	0.471
Model 2	0.09	-0.13, 0.32	0.404	0.12	-0.13, 0.37	0.335
Gestational weight gain						
Model 1	6.07	-2.60, 14.8	0.167	6.58	-2.75, 15.9	0.163
Model 2	2.11	-7.15, 11.4	0.650	4.45	-5.73, 14.63	0.385
Sleep						
Model 1	0.22	-0.89, 1.33	0.694	0.04	-1.15, 1.24	0.943
Model 2	0.39	-0.70, 1.48	0.475	0.10	-1.11, 1.31	0.869
PPBMI						
Model 1	-0.06	-0.27, 0.16	0.593	-0.02	-0.26, 0.21	0.837
Model 2	0.04	-0.19, 0.27	0.715	0.07	-0.18, 0.33	0.562
Glucose screen						
Model 1	0.03	-0.02, 0.07	0.209	0.03	-0.02, 0.08	0.187
Model 2	0.04	-0.01, 0.08	0.081	0.04	-0.01, 0.09	0.111

Model 1 presents unadjusted results. *Model 2* is adjusted for maternal age and smoking status

Appendix B Supplemental Figures



Supplemental Figure 1. Mean (SD) Cardiovascular Health Component Scores Averaged Across Gestation, by Study



Supplemental Figure 2. Association Between MVPA and FV% (Quadrant protocol) in Third Trimester, Overall (a) and by Study (b)

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