VASCULAR REMODELING ASSOCIATED WITH PREGNANCY

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Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2014

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

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December 2, 2014

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University of Pittsburgh, 2014

ABSTRACT

Objectives: Research indicates a relationship between pregnancy and cardiovascular disease, but the cause of this relationship is unknown. One possible explanation is that there is a relationship between pregnancy and vascular change. The objective of this dissertation is to illuminate this relationship by exploring 1) the association between parity and structure of the carotid arteries in a population of overweight or obese women of reproductive age and 2) the normal course of common carotid artery (CCA) remodeling and changes in stiffness of the brachial artery throughout a healthy first pregnancy and postpartum.

Methods: The first paper provides a cross-sectional analysis of the relationship between reproductive factors and structural measures of the carotid artery in overweight and obese young women participating in the Slow Adverse Vascular Effect of Obesity (SAVE) clinical trial. The subsequent 2 papers provide results of the Maternal Vascular Adaptation to Healthy Pregnancy (MVP) study, in which 43 healthy young women were assessed prospectively throughout their first pregnancies with ultrasounds of their carotid arteries, a measure of brachial artery distensibility, and physical and metabolic measures.

Results: In the SAVE study, nulliparous women had greater common carotid inter-adventitial diameter (IAD) and mean CCA intima media thickness (IMT) compared with parous women

after adjustment for age, race, and CVD risk factors. In the MVP study, after adjustment for age and pre-pregnancy BMI, mean IAD increased each trimester and returned to baseline postpartum. Mean CCA IMT was increased postpartum compared to 1st and 2nd trimester values. Mean brachial artery distensibility decreased from 1st trimester to 3rd trimester and then remained unchanged postpartum.

Conclusions: Among overweight and obese young women, nulliparity was associated with less healthy carotid arteries. During the course of healthy first pregnancy, some negative vascular changes (greater CCA IMT and stiffer brachial arteries) occurred that persisted into the postpartum period.

Public Health Significance: Cardiovascular disease is the leading cause of death in women. Early identification of women at high risk (nulliparous) offers early opportunity for risk reduction. Understanding normal vascular changes of pregnancy may help explain the pathophysiology of preeclampsia, the cause of 50,000 maternal deaths per year.

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PREFACE

No work of this size could be accomplished without the help of many people. I would like to thank Kim Sutton-Tyrrell, my advisor my first 2 years at the University of Pittsburgh, for her encouragement and skilled teaching. I try to use her pedagogical techniques to help my own students as she helped me. I greatly appreciate how all the members of my committee, especially Emma Barinas-Mitchell and Janet Catov, strode in to fill Kim's shoes when it became necessary. Candace McClure designed and funded the MVP study, without which this work would not exist. Eileen Cole and Laura Kinzel taught me everything I needed to know about the nuts and bolts of working with study participants, and I owe the high retention rate in the MVP study to their good instruction. Kathleen D'Aurelio, Antoinette Marasco, and Marina Bodomova at the University of Pittsburgh Ultrasound Research Laboratory were endlessly flexible at accommodating my needs and the needs of study participants, and I am very grateful.

I would like to thank my fellow cardiovascular trainees for their assistance and support, in particular Timothy Hughes, who was a patient mentor, Andrew Althouse, who taught me to transpose datasets, and my officemates, Jennifer Cooper, Carrie Hanley, and Jessica White, who kept me inspired and supplied with chocolate.

My dissertation research and training was supported by an NICHD training grant (5T32HD55162-5) and an NHBLI training grant to the University of Pittsburgh (T32HL083825). The SAVE clinical trial was supported by grant R01 HL077525 from NHLBI to the University of Pittsburgh, and the MVP study was supported by the Department of Epidemiology Small Grants Program.

I dedicate this work to the women of MVP, who despite being exhausted, nauseous, and hypoglycemic, loyally came to early morning study visits in the hopes that their participation would help prevent pregnancy complications for women in the future, and to Myron and Peggy, my evening and morning stars.

1.0 INTRODUCTION

There is no more profound example of vascular remodeling, or lasting structural changes in blood vessel walls, than that which occurs in the uterine vasculature during the course of a healthy pregnancy. The changes which occur allow the vasculature to nourish the growth of a healthy, full-term baby while maintaining the wellbeing of the pregnant woman. When this uterine vascular remodeling occurs incorrectly it can result in pregnancy complications such as preeclampsia[1], preterm birth[1,2], intrauterine growth restriction[3-5], and stillbirth[6]. While there is significant understanding of the changes that occur in the uterine vasculature, much less is known about changes that occur in the systemic arteries of pregnant women. Systemic arteries and uterine vasculature both remodel in response to similar stimuli of hemodynamic stressors, and hormonal and metabolic influences. Thus, a better understanding of the effects of pregnancy on the systemic arteries may contribute to improved detection of women at risk for pregnancy complications before or early in pregnancy, help explain racial disparities in birth outcomes, and help explain the differences in cardiovascular risk found in women of different parity.

1.1. VASCULAR REMODELING AND VASCULAR STIFFNESS

1.1.1 Definitions

Blood vessels change size to accommodate acute and chronic changes in the body's metabolic needs. Acutely, blood vessels can dilate to bring increased blood flow to an organ, such as to accommodate the needs of skeletal muscle during exercise, or constrict, such as to conserve body heat in the cold. In contrast, when there are long-term changes to the demands on the blood vessels they can remodel.[7] Vascular remodeling is lasting structural change in blood

vessel walls in response to hemodynamic, metabolic or hormonal stimuli.[8] Size of the vessel wall can increase through hypertrophy or hyperplasia of vascular smooth muscle cells, and decrease through hypotrophy and apoptosis. These structural changes are distinct from the functional changes of dilation or constriction.[8]

The goal of vascular remodeling is to maintain homeostasis of the different hemodynamic stresses on the vessel. When this is achieved it is termed adaptive remodeling; when homeostasis is not achieved the remodeling is maladaptive.[9] Maladaptive vascular remodeling is seen in states of ill-health including hypertension, metabolic syndrome,[9] chronic kidney disease,[10] and rheumatoid arthritis.[11]

A related concept to vascular remodeling is vascular stiffness, or the elastic properties of the vessel wall.[12] Vascular stiffness depends both on structural properties of the vessel such as wall thickness and composition and functional properties such as changes in diameter and endothelial function.[13] Reflection of these two components allows vascular stiffness to be viewed as a summary measure of vascular health.[14]

1.1.2 Assessment of Vascular Remodeling

The arterial wall is comprised of 3 layers: the intima, the media and the adventitia. The inner layer is the intima, which consists of a single layer of endothelial cells on a thin extracellular matrix. The intima separates the blood from the structural part of the vessel, and regulates flow of nutrients and blood components.[15] It is also metabolically active and involved with hemostasis, inflammation and angiogenesis, and is the site of atherosclerotic plaque formation. The middle layer, or media, is the main structural component of blood vessels and is formed of smooth muscle cells surrounded by an extracellular matrix of collagen, elastic fibers,

fibroblasts, and an internal elastic lamina.[15] The adventitia is the outer layer of the blood vessel and is a network of connective tissue, elastin, collagen, nerves and blood vessels.

This dissertation primarily concerns remodeling of the carotid artery. The paired common carotid arteries (CCA) are the main source of blood flow to the head. While paired, the arteries are not completely symmetrical. The left common carotid artery branches directly off the aorta and the right common carotid branches off the brachiocephalic artery, which originates in the aorta.[16] Both common carotid arteries widen into the carotid bulb and divide into the internal carotid artery (ICA), carrying blood to the brain, and the external carotid artery, carrying blood to the scalp and face.[16]

While many modalities can be used to image the carotid artery, this dissertation focuses on ultrasound assessment. Ultrasound assessments of the carotid artery are highly reproducible, valid, and used frequently in epidemiologic research studies and increasingly in clinical practice.[17] The Ultrasound Research Laboratory at the University of Pittsburgh uses B-mode ultrasound with high frequency linear array transducers to image the carotid arteries for measurement of intima-media thickness (IMT), the thickness of those 2 wall layers, and inter-adventitial diameter (IAD), the distance from near wall adventitial medial-interface to far wall adventitial-medial interface. Images are digitally recorded and read with an automated reading system.[18] This modality can also assess presence and extent of plaque formation.

1.1.3 Assessment of Arterial Stiffness

Arterial stiffness can be measured along any branch of the arterial system. Aortic, or central, stiffness is most commonly assessed, but peripheral stiffness can be assessed in the arteries of the arm and leg. There are many indices of arterial stiffness, including elastic

modulus, Young's modulus, arterial distensibility, arterial compliance, pulse wave velocity, augmentation index, and stiffness index.[19] These can be assessed with ultrasonography, magnetic resonance imaging, or pressure waveform analysis.[19] Studies of arterial stiffness in pregnancy have most often utilized pressure waveform analysis to measure augmentation index, the difference between the first and second systolic peak divided by pulse pressure, and pulse wave velocity (PWV), the speed of travel of the pulse wave along an artery, measured as distance over time.[19] Both greater augmentation index and pulse wave velocity are associated with greater arterial stiffness.

This dissertation is primarily concerned with measurement of brachial artery distensibility. Distensibility is the relative change in arterial diameter for a given change in pressure.[19] In the Maternal Vascular Adaptation to Healthy Pregnancy Study we utilized the DynaPulse 2000A system to derive brachial artery distensibility from analysis of the pressure waveforms in the brachial artery. This technique has been found to be reliable when compared to invasive monitoring techniques.[20]

1.1.4 Significance of Intima Media Thickness and Adventitial Diameter

IMT and IAD are both markers of several dimensions of arterial health, with greater diameter and thickness representing less healthy vessels. IMT is thickened in the presence of atherosclerotic plaque, but IMT is much more than a marker of plaque and can thicken without its presence.[21] Both IMT and IAD tend to increase with age,[22] on average doubling to tripling between 20 and 90 years of age,[21] but there is wide variation in this increase between different individuals and cultures. IMT and IAD mark cumulative exposure to cardiovascular risk factors, and are increased by many of the same factors that cause atherosclerosis. Similar physiologic

processes lead to thickened IMT and atherosclerosis.[21] Thickened IMT and IAD also can become risk factors for cardiovascular disease, because thickened, distended vessels are less capable of responding effectively to changes in blood pressure,[23] and thickened arteries are most prone to the development of atherosclerosis.[13] Thus, IMT and IAD are measures of arterial health because they describe extent of plaque and cardiovascular risk factor burden, but also because they are their own innate measures of vascular health.

Many studies document the relationship between IMT and IAD and traditional cardiovascular risk factors. Greater IMT is found with male sex,[22,24] greater systolic blood pressure,[22,24,25] cigarette smoking,[22,24,25] higher total cholesterol,[22] higher LDL-c cholesterol,[24,25] higher triglycerides,[22] and higher blood glucose.[22] Greater IAD is found with male sex,[26,27] black race,[26] increasing weight,[26,27] greater height,[27] higher blood pressure,[27] higher blood glucose,[27] alcohol use,[27] higher triglyceride levels,[27] and lipid-lowering medication use,[26] and negatively associated with HDL-c concentrations.[26,27] All these relationships are in the direction one would expect if the traditional cardiovascular risk factors affect IMT and IAD.

Intima-media thickness and IAD both independently predict cardiovascular disease risk beyond the mere presence of traditional cardiovascular risk factors. Greater IMT is related to higher risk of myocardial infarction,[28-32] coronary artery disease endpoints,[33-35] and cerebrovascular disease endpoints.[30,33,36-38] Greater IAD is related to higher risk of cerebrovascular disease,[33] and incident cardiac events.[39] Assessment of IMT and IAD thus provides assessment of vascular health and levels of cardiovascular risk factors, and assists in prediction of future health outcomes.[17]

1.1.5 Significance of Arterial Stiffness

Greater arterial stiffness (or lower compliance and distensibility) is a marker of arterial aging and less healthy vasculature. Both structural and functional arterial changes lead to increased stiffness.[14,21] Structural changes include thickened arterial wall, increased collagen content, fragmentation of elastin, and calcification.[21] Functional changes include decreased endothelial function.[21] Greater arterial stiffness is associated with known cardiovascular risk factors including greater age,[21,40] obesity,[40] sodium intake, [14,41] abnormal glucose metabolism,[14,41] chronic renal disease,[14] and hypertension,[41-43] and is associated with greater coronary heart disease,[44-46] stroke,[44] cardiovascular mortality,[44,46] and all-cause mortality.[44-46]

Stiffness can be measured in any arterial bed but is most often assessed in the central aorta and carotid, brachial and femoral arteries. Aortic stiffness has traditionally been viewed as of the greatest importance because it contributes to the development of isolated systolic hypertension, congestive heart failure, and inadequate perfusion of the coronary arteries.[12,14] Measures in the peripheral arteries (brachial and femoral) have often been seen merely as reflecting central stiffness.[47] More recent research supports examining the different arterial beds separately. Arterial stiffness is lowest in the central bed (aorta) and higher in peripheral arteries (brachial and femoral).[12] Anatomically the vessels differ, with the aortic wall primarily elastic, the brachial artery wall more muscular, and the femoral artery a combination of the two.[48] Risk factor association differs for different regions. Compared to peripheral stiffness, central stiffness is more associated with weight/body mass index.[49] Peripheral stiffness is more associated with age and glucose metabolism.[49,50] A recent study demonstrated differences in the ability of stiffness in different arterial beds to predict outcomes.[45] It found that greater carotid and

femoral stiffness were associated with cardiovascular events and all-cause mortality, while lower brachial stiffness predicted events and mortality only in people with insulin resistance.[45]

1.2 IMPORTANCE OF UNDERSTANDING VASCULAR CHANGES OF PREGNANCY

1.2.1 Pregnancy Complications with Vascular Components

Many pregnancy complications have a vascular component. The spiral arteries of the uterus, which carry blood to the placenta, are remodeled during pregnancy by systemic factors and invasion of trophoblast cells to become greatly enlarged, and bring large amounts of blood to the placenta at low pressure. Failure of this remodeling is a precipitating factor for the development of preeclampsia, preterm birth, and intrauterine growth restriction.[1-5,51,52] Of interest, failure of spiral artery remodeling is not sufficient to cause disease. Approximately half of women who have inadequate remodeling, as demonstrated by abnormal uterine artery blood flow measured by Doppler ultrasound at about 20 weeks of gestation, do not develop pregnancy complications.[53] Many women with abnormal uterine artery blood flow do not exhibit abnormal spiral artery remodeling on placental biopsy.[54]

Preeclampsia is a pregnancy-specific disease complicating 2-8% of pregnancies,[55] defined by new origin hypertension and proteinuria occurring after 20 weeks of pregnancy. It is a multi-system disorder that can also include liver damage, kidney damage, hematological changes such as hemolysis and destruction of platelets, neurological symptoms such as headaches and seizures, and intrauterine growth restriction for the fetus. While the cause of preeclampsia is not fully understood, there appear to be three stages: a maternal predisposition to abnormal placentation and failed spiral artery remodeling ,the abnormal placentation and failed

spiral artery remodeling themselves, and then pathologic maternal response to the failed remodeling.[53] Maternal factors that may both predispose to abnormal placentation and increase likelihood of developing preeclampsia once the abnormal placentation has occurred may include factors such as endothelial dysfunction, insulin resistance, obesity, preexisting hypertension, dyslipidemias, lifestyle and genetic factors.[52,53]

There is no effective treatment for preeclampsia except delivery. The whole system of prenatal care in the developed world is largely designed to facilitate early detection of preeclampsia, and maternal deaths from preeclampsia in the US are relatively rare. Preeclampsia contributes to 16% of maternal deaths in developed countries and up to 23% of maternal deaths in Latin America.[56] Most of the 50,000 maternal deaths each year from preeclampsia occur in the developing world, where women do not have access to prenatal care or labor induction to treat developing preeclampsia.[57] Preeclampsia also contributes to perinatal mortality and morbidity, and contributes to 12% of growth restricted and up to 20% of preterm infants.[57]

Preterm birth is any birth before 37 completed weeks of pregnancy. There are many causes of preterm birth including infection/inflammation, trauma and maternal or fetal medical indications.[58] Failed spiral artery remodeling appears to occur in about 1/3 of preterm births.[1,2,59] Preterm birth occurs in approximately 12% of US births.[60] Babies born preterm have disproportionately greater morbidity and mortality. Compared to babies born full term, babies born at 34-36 weeks gestation have 3.5 times greater mortality, and those born even earlier have a 50 times greater mortality.[60] Babies born prematurely have higher risk of lifelong health and neurodevelopmental problems.[61]

Fetal growth restriction is diagnosed when a fetus or newborn has a weight below the 10th percentile for its gestational age.[62] Estimates of prevalence, morbidity, and mortality for growth

restricted babies are difficult because growth restriction cannot be differentiated from prematurity in many large population studies, and the National Center for Health Statistics does not separate out growth restricted from other small babies in its birth data.[60] Growth restricted babies have higher mortality and long term neurodevelopmental impairment than normally grown babies.[63] Abnormal placentation is a known cause of growth restriction.[3-5]

In the United States approximately 1 in 160 births results in stillbirth, or fetal death after 20 weeks of pregnancy.[6] Evidence of vascular maladaptation is less clear than with the other pregnancy complications discussed, but a study supports that defective first trimester placentation is associated with stillbirths.[64] A population-based study of stillbirth in the United States found that placental disease was a probable/possible cause of 22% of stillbirths, and that umbilical cord abnormalities were a probable/possible cause of 13%.[6]

1.2.2 Pregnancy's Association with Changes in CVD Risk Factors

Pregnancy is associated with life-long changes in CVD risk factors. Most studies on the topic are cross-sectional in design and cannot fully assess temporality, however existing data suggest that some changes seem to accumulate with each pregnancy, some are one time changes with first births, and for others little is known.[65,66] Many of these changes are related to metabolism and would be expected to have a negative effect on cardiovascular health. A large study using NHANES data in the US found a linear relationship between parity and prevalence of metabolic syndrome. In all women except non-Hispanic black women each additional live birth was associated with 13% higher odds of having metabolic syndrome.[67] Parity was associated with greater waist circumference, lower HDL-c and higher triglyceride concentrations, but not with blood pressure or glucose concentrations. Similar results were

found in a Chinese sample, except this study also found higher fasting glucose concentrations with each birth.[68] It seems likely that changes in body fat composition including increased waist to hip ratio and abdominal adiposity are related to these changes.[69-71] Visceral abdominal fat is believed to increase insulin resistance more than peripheral fat via mechanisms including higher lipolysis, leptin resistance, and inflammatory cytokines, and lower adiponectin levels.[72] A study on the relationship between parity and inflammatory markers found no relationship with 17 inflammatory markers.[73]

A prospective study in the US suggests that some CVD risk factors demonstrate a "first birth effect" with long-lasting one-time changes after first births which are not repeated after subsequent births. These include a decline in HDL-c of 3-4 mg/dl,[65] and paradoxically, the beneficial change of a 2 mm Hg drop in systolic and diastolic blood pressures after a first birth among women with a non-hypertensive pregnancy.[66] LDL was found to drop about 4 mg/dl after a first birth, but this difference was not statistically significant after accounting for changes in smoking status, months of oral contraceptive use, education, body weight, waist circumference, physical activity and alcohol intake.[65]

Understanding how pregnancy affects CVD risk factors may allow for interventions during pregnancy and postpartum to mitigate the negative effects of pregnancy on CVD risk factors.

1.2.3 Pregnancy as a CVD Risk Factor

Pregnancy itself may be a CVD risk factor. Studies of the relationship between gravidity or parity and cardiovascular disease have had varied results,[74] but the largest studies show greater risk of CVD for women of high parity.[75-78] The largest population-based cohort study

followed 1.3 million Swedish women for 9.5 years, and found a j-shaped relationship between parity and CVD events.[77] Women with parity of 2 have the lowest CVD risk. Nulliparous and primiparous women both have about a 10% higher risk. Risk then increases with parity, so that women with 5 or more births have a more than 50% greater CVD risk than women with 2 births. The authors postulate that higher risk in women of low parity is secondary to confounding from subfertility in that population. Subfertility has many possible causes, one of which, an elevated ratio of androgens to estrogens, may plausibly be associated with CVD risk.[79] Greater risk in women of high parity may be related to accumulation of CVD risk factors with each pregnancy. A significant difficulty with studies of parity is that parity is closely connected to various socio-economic factors that may also be associated with CVD risk, and which are difficult to control for in research studies.[80] A recent study of 500 very homogeneous Old Order Amish women with mean parity of 6.7 found no relationship between parity and CVD, even though higher parity was associated with greater BMI.[81] This suggests that residual confounding due to socio-economic factors may be a cause of the greater CVD risk found in women of higher parity.

Studies of examining number of children and cardiovascular disease risk in men illuminate this relationship. A US study found a U-shaped relationship between number of children and cardiovascular mortality with the nadir at 2 children; after adjustment for socio-economic risk factors only men with no children or one child continued to have greater risk.[82] A study comparing men with and without children also found greater risk of mortality from ischemic heart disease for childless long-term cohabiting men.[83] Intima media thickness has not been shown to vary by number of children in men[84] and may decrease with the birth of a child.[85] These findings suggest that subfertility may be associated with cardiovascular disease in men as

well as in women, and that higher incidence of cardio-vascular disease for men with more children might be due to confounding from socio-economic status.

1.3 STIMULI FOR VASCULAR REMODELING AND THE EFFECTS OF PREGNANCY

Stimuli for vascular remodeling include hemodynamic, hormonal and metabolic factors. All of these factors are affected by pregnancy in ways that should influence vascular remodeling.

1.3.1 Hemodynamic Changes

Arterial walls attempt to maintain homeostasis between the two main stresses from blood flow: shear stress and tensile stress. Shear stress is the tangential or frictional force of blood flowing along the intima of the artery. The adaptive response to shear stress is for the diameter of the blood vessel to increase.[7,8,86] Tensile stress is the force of blood perpendicular to the wall of the artery. The adaptive response to tensile stress is for the wall thickness to increase.[7,87] In the healthy individual diameter and wall thickness change in concert to normalize stress in response to flow changes. Typically, increased blood flow leads to increased diameter. This then increases tensile stress, which leads to thickening of the vessel wall, maintaining homeostasis.[23] Wall thickening increases arterial stiffness.[21]

Pregnancy involves massive hemodynamic changes that increase shear stress on the blood vessels (Table 2.1). Cardiac output increases by early in the first trimester of pregnancy[88]

peaking at 30-60% above the non-pregnant level in the late 2nd or early 3rd trimester.[88-91] The increase is caused by elevation of plasma volume, which increases by about 35-45%,[88,89,91,92] leading to increased stroke volume[88,89] and also from an increased heart rate of about 10-15 beats per minute.[93-95] Some studies show that cardiac output remains elevated 1 year postpartum.[90] Greater increases in blood volume are found in women of higher BMI, higher parity, with larger fetuses, and with multiple gestations.[92] While mechanisms of the increased plasma volume are not fully understood, it is theorized that fetal dehydroepiandrosterone sulfate **(**DHEAS) production stimulates the renin-angiotensin-aldosterone system to retain sodium and water.[96]

Pregnancy is also characterized by decreased systemic vascular resistance. This decreases by the 8th week of pregnancy[88] and reaches a nadir of about a 35% decrease in the 2nd trimester.[89,93,94] It remains below baseline 1 year postpartum.[90] Following this decrease, blood pressure also decreases in early pregnancy. There is a 10% decrease in mean arterial pressure by the 8th week of pregnancy, primarily driven by decreased diastolic pressure. The nadir of blood pressure occurs during the second trimester, and blood pressure increases to baseline at term.[88,90,91,94,97] Mechanisms for decreased systemic vascular resistance are theorized to include increased concentrations of vasodilatory prostanoids such as prostacyclin,[98] increased concentrations of nitric oxide,[99] vasodilatory effects of the renin-angiotensin-aldosterone system,[98] the development of the low-resistance placental bed,[88] and local vasodilation in response to heat produced by the fetus.[88] The combination of increased blood volume and decreased systemic vascular resistance increases the velocity with which blood passes through the vasculature. This in turn increases the shear stress on the blood vessels.

1.3.2 Reproductive Hormones

An increasing body of research, much of it done in mid-life or older women, suggests that reproductive hormones play a role in vascular remodeling. Use of menopausal hormone therapy has been shown to be independently associated with thinner CCA IMT,[100] ICA IMT,[101] CCA adventitial and lumen diameter,[102] and greater arterial compliance.[103,104] CCA IMT and lumen diameter have been found to be greater in post- than pre-menopausal women,[105] suggesting that estrogen has a protective effect. The most comprehensive study found no relationship between endogenous estradiol concentrations and baseline IMT or IMT progression in middle-aged women, but did find that higher estrogen levels were associated with lower baseline adventitial diameter,[106] and slower progression of adventitial diameter over time.[107] In general, arterial stiffening increases after menopause, suggesting a hormonal link.[103,104,108] Additionally, higher testosterone concentrations are associated with thicker CCA IMT in women in some studies,[109,110] but not others.[111-113] Sex hormone binding globulin has been found to be negatively associated with IMT.[107,111,114]

Direct mechanisms by which reproductive hormones may affect vessel walls are unclear. Vessel walls express receptors for estrogen, progesterone and testosterone.[115] Estrogen appears to have anti-inflammatory effects on blood vessel walls.[116] Reproductive hormones might also affect the connective tissue component of blood vessel walls. In vitro, aortic smooth muscle cells incubated with estradiol, progesterone, and testosterone all had lower collagen deposition than cells incubated with a control solution. Elastin concentrations were higher in the cells incubated with estradiol and progesterone.[117] Lower collagen and higher elastin concentrations

would be expected to produce less stiff arteries. Also in vitro, estrogen appears to inhibit vascular smooth muscle cell proliferation, which could decrease atherosclerosis.[115]

Pregnancy induces dramatic increases in concentrations of sex hormones. Estrogen concentrations increase more than 100-fold in pregnancy.[118] Estrogen is initially produced maternally by the ovaries, adrenal glands, and peripheral conversion of androgens to estrogens.[119] Estrogen production is then assumed by the placenta.[119] Progesterone concentration increase hundreds of times throughout the course of pregnancy.[118] Progesterone is produced initially by the maternal corpus luteum; by about 10 weeks of pregnancy the placenta assumes production of progesterone and the corpus luteum degrades.[119,120] Total testosterone concentrations increase throughout pregnancy; free testosterone concentrations remain stable initially, but approximately double in the third trimester.[121] Human chorionic gonadotropin, a hormone unique to pregnancy, is produced by the trophoblast cells by 8 days after conception, peaks at 8-12 weeks of pregnancy, and then is present at lower concentrations throughout gestation.[118] Additionally, maternal cortisol concentrations roughly double during pregnancy.[122] These hormonal changes plausibly can affect the vasculature.

1.3.3 Metabolic Changes

An increasing body of research shows that metabolic factors are related to IMT, intra-adventitial diameter, and arterial stiffness. Greater IMT and/or IAD have been shown to be independently associated with treated diabetes mellitus,[26] glucose concentrations,[123-126] and insulin resistance.[125] Some research suggests that spikes in glycemic concentrations may be more important than total blood glucose concentrations in affecting IMT.[123,127] There are several mechanisms by which higher blood glucose concentrations affect vessel walls. They increase

oxidative stress,[127] affect the structure of extracellular matrix proteins, and reduce bioavailability of NO produced by the endothelium.[128] Impaired glucose metabolism is also linked with arterial stiffness, largely due to increased collagen cross-links in the vessel wall caused by glycation.[14,21] Lipid concentrations also affect the vasculature both by promotion of atherosclerosis and through direct effects. Greater IMT and adventitial diameter have been shown to be greater in people with lower HDL,[26,125,126,129] higher total cholesterol,[25,26,126] higher LDL,[24,86,105,125] and lipid-lowering medication use.[26] Inflammation is related to greater arterial stiffness because it leads to a higher ratio of collagen to elastin in the vessel wall.[14]

Pregnancy is accompanied by atherogenic metabolic changes (Table 2.1). While insulin sensitivity may be slightly increased in early pregnancy, as pregnancy progresses insulin resistance develops. Mean glucose concentrations are similar in pregnant and non-pregnant women, but pregnancy is characterized by slightly lower fasting values, higher post-prandial values, and 3 times higher insulin concentrations.[130] Lipid concentrations dip slightly in early pregnancy, and then increase dramatically. The greatest increase is in triglyceride concentrations, but total cholesterol, LDL-c, and HDL-c also increase.[131] Pregnancy is an inflammatory state, with elevated concentrations of C-reactive protein,[132] platelet activation,[133] most blood clotting factors,[134] and activated neutrophils.[135]

1.4 UTERO-PLACENTAL VASCULAR REMODELING IN PREGNANCY

1.4.1 Uterine Artery Remodeling

Arterial remodeling is evident in the arteries supplying the uterus. These arteries undergo dramatic changes as blood flow to the uterus increases from 20-50ml/min in the non-pregnant state to 450-800m/min in a singleton pregnancy. [136,137] In response, the diameter of the uterine artery approximately doubles,[136,138] and diameters of the other arteries increase as well, an example of outward hypertrophic remodeling. It appears that vascular smooth muscle cell increase in both number and size to accomplish this remodeling.[138]

While not fully understood, mechanisms behind this remodeling seem to be both systemic and localized and follow general principles of vascular remodeling. Systemic influences include the increased concentrations of estrogen and progesterone in pregnancy (indeed, uterine artery blood flow increases from day 10 of the menstrual cycle).[122,139] Several lines of experimental research suggest that this is the first instigator of change.[138] As pregnancy progresses, localized mechanisms including shear stress and presence of growth factors produced by the placenta such as vascular endothelial growth factor may be responsible. Both hormonal and shear stress mechanisms may affect arterial diameter by up-regulating endothelial nitric oxide. Estrogen and progesterone both affect growth factor secretion[136] and estrogen stimulates DNA synthesis and mitosis in uterine radial arteries.[138]

1.4.2 Spiral Artery Remodeling

Even more profound remodeling is seen in the changes that occur in spiral arteries, the last maternal vasculature before the placenta. These arteries become greatly dilated and lose smooth muscle, allowing them to bring modestly increased amounts of blood at low pressure to the placenta.[118]

Remodeling of the spiral arteries likely begins with the same stimuli as other remodeling – the increased reproductive hormone concentrations of pregnancy, including human chorionic gonadotropin, estrogen and progesterone, and increased shear stress.[118,140,141] But the principle mechanism of this remodeling is unique – invasion of spiral arteries by extravillous trophoblast cells (EVT). The EVT leave the placental villi and invade the decidual stroma, colonizing the spiral arteries both from the outside (interstitial invasion) and by migrating inside the lumen of the vessels (endovascular invasion.)[142] The vessel endothelium and vascular smooth muscle cells are replaced by the EVT.[136]

The effect of metabolic factors on spiral artery remodeling is more difficult to determine. Both blood glucose and lipid concentrations may be involved. It is known that women with diabetes have higher incidence of pregnancy complications involving inadequate spiral artery remodeling than do women without diabetes, suggesting that hyperglycemia may play a role.[143] Small studies have found higher incidence of poor spiral artery remodeling in diabetic than in non-diabetic women [144] and in diabetic women with worse glycemic control.[145] Lipids might also be involved in spiral artery remodeling. The relationship between lipid concentrations and preeclampsia has not been fully elucidated. Some studies show elevated pre-pregnancy lipid concentrations in women who will develop preeclampsia[146-148] and there is clear evidence of

higher pregnancy lipid concentrations in women who will develop preeclampsia.[131,149] Spiral arteries that remodel incompletely contain lipid deposits resembling atherosclerosis.[150,151] It is unclear whether the lipid abnormalities contribute to inadequate remodeling of spiral arteries, or if abnormally remodeled arteries are sensitive to the effects of elevated lipid concentrations.[151]

1.5 STUDIES OF SYSTEMIC VASCULAR REMODELING ASSOCIATED WITH PREGNANCY

1.5.1 Anatomic Data

Few studies have examined structural changes in systemic arteries during pregnancy, but those that exist suggest that there are substantial changes. Histologic studies have found that in guinea pig carotid arteries there is progressive thinning of the intimal and medial layers throughout pregnancy, caused by hypotrophy of both smooth muscle and endothelial cells,[152] while the aorta demonstrates only a thinning of the intima, again associated with endothelial hypotrophy.[153] In contrast, guinea pig femoral arteries showed no change.[154] Human studies have found that the aorta has greater diameter and is more elastic during pregnancy.[155]

1.5.2 Cross-Sectional Studies of IMT in Healthy vs. Complicated Pregnancies

A number of small cross-sectional studies compared IMT in healthy and complicated pregnancies (Appendix 1, Table 6.1). Studies showed that women who had pregnancies complicated by preeclampsia had greater CCA IMT at 3-13,[156] and 12-24[157] months, and greater mean IMT 25 years[158] postpartum than women with uncomplicated pregnancies, after adjustment for traditional cardiovascular risk factors. Other studies showed no difference in CCA IMT between women with preeclampsia and women with normal pregnancies at 5[159] or 7 years postpartum.[160] A study comparing women at high risk for development of preeclampsia because of elevated uterine artery pulsatility indices at 20-23 weeks of gestation to women with normal indices found no difference in mean CCA IMT, but significantly greater mean ICA IMT in women with the at-risk pregnancies.[161] A study that compared women with preeclampsia at term

to women with uncomplicated pregnancies found greater CCA IMT and internal diameter in the women with preeclampsia.[162]

Several studies compared women with gestational diabetes to women with non-diabetic pregnancies (Appendix 1, Table 6.2). One found that women with gestational diabetes had greater CCA IMT at 25 weeks of pregnancy,[163] and another that the mean of CCA and ICA IMT was greater in women with gestational diabetes in both the 2nd and 3rd trimesters.[164] At 3 years postpartum a small study found no difference in CCA IMT when women were matched for BMI[165] but a larger, age-matched sample from the same group did find greater CCA IMT in the women with gestational diabetes at 3 years postpartum.[166] Other studies found significantly thicker IMT (mean of all segments) in women with gestational diabetes at 2-3 years postpartum,[167] and at 6 years postpartum (mean of CCA and ICA)[168] but not statistically significantly greater at 4 years postpartum.[169] These women had IMT comparable to a comparison group with metabolic syndrome. A study dividing women with gestational diabetes into those with more mild disease not requiring insulin and more severe disease requiring insulin found greater CCA IMT at 3 months postpartum only in women who required insulin.[170]

The preponderance of research on this topic suggests that women with pregnancies complicated by preeclampsia or gestational diabetes may have greater IMT and possibly CCA diameter during pregnancy and postpartum than women with normal pregnancies. The cross-sectional nature of these studies makes it impossible to assess temporality. Is greater IMT a marker for women who are at risk of pregnancy complications? Or do the pregnancy complications themselves cause the greater IMT? Or does a process that goes wrong in pregnancy create both the complication and the greater IMT? Longitudinal studies are needed to answer these questions.

1.5.3 Prospective Studies of IMT in Pregnancy

Prospective data on changes in the carotid artery throughout the maternity cycle are limited. One study followed 23 women throughout their pregnancies and 11 into the postpartum period, and found that CCA IMT and diameter both increased slightly from the first to the third trimester, and then decreased at 3-6 months postpartum, although remaining above baseline measures. The only significant difference was in IMT between the 1st and 3rd trimesters.[171] A second study by the same group of researchers followed 12 women and found both CCA IMT and diameter increased throughout pregnancy and decreased postpartum, although the differences were only significant for diameter.[172] Another group found no difference in the mean of the CCA and ICA IMT between the 2nd to 3rd trimesters in either healthy pregnancies or pregnancies complicated by gestational diabetes.[164] A study that measured IMT at 2 time points 6 years apart found an increase of mean and maximum CCA IMT in women for each child born during the time period.[85] While prospective data is lacking, it does suggest that pregnancy results in increased carotid IMT and diameter, which recedes postpartum, although not back to pre-pregnancy measures.

1.5.4 Intima vs. Media

Two studies assessed the carotid wall during pregnancy using extremely high-frequency ultrasound imaging that can differentiate the intima from the media. A cross-sectional study using this method found that while IMT was identical in women at 39 weeks of pregnancy and a healthy non-pregnant control group, the pregnant women had significantly thinner intimas and thicker medias.[173] In contrast, a recent study utilizing this method to assess women during each

trimester of pregnancy and at a year postpartum found changes neither in CCA IMT throughout pregnancy, nor in the individual components or ratio of intima to media. All of these decreased significantly at 1 year postpartum.[174] This was the only study to examine predictors of IMT in pregnancy. It found that lower concentrations of serum estradiol, older age, higher BMI and blood pressure were associated with thicker intima, thinner media, and higher ratio of intima to media. The authors state that a higher intima to media ratio is associated with atherosclerosis, and that it is logical that these traditional cardiovascular risk factors are related to the ratio in pregnant women.

These studies cannot be compared to the rest of the literature. This ultrasound technique has been used primarily by one research group and has not been validated against the more standard technique. In many cases, investigators using this protocol image only the near wall of the common carotid artery, whereas the standard technique images near and far wall, or far wall only (a more valid and reliable measure of IMT), and also takes fewer measurements than the standard protocol of imaging intima and media together.

1.5.5 Cross-Sectional Studies of Parity and IMT in Midlife and Older Women

Three cross-sectional studies examine the relationship between parity and IMT in midlife and older women. Two found greater CCA IMT with higher parity after adjustment for demographic and traditional cardiovascular risk factors.[175,176] One of these also found thicker IMT in nulliparous women[176] while the other did not. The third study found no relationship between parity and the mean of CCA and bulb IMT.[177] These differences may be explained by the different measures used. The studies measuring CCA IMT found thicker IMT with higher parity, while the study measuring the mean of CCA and bulb IMT did not. IMT in the different

carotid regions reflects somewhat different processes, with bulb IMT more reflective of lipid concentrations and atherosclerosis and CCA IMT more reflective of arterial aging. The two studies measuring CCA IMT differed significantly in the percentage of nulliparous women in their samples. The Study of Health in Pomerania, which showed greater IMT in nulliparous women, had 8.5% nulliparity, while the Rotterdam Study, showing no effect, had 20% nulliparous women. The authors suggest that the nulliparous women in the Pomerania Study may represent women with true infertility, as 8.5% is close to the figure of infertility in the general population, and that their greater IMT may be due to atherogenic factors that accompany some infertility such as higher androgen and glucose concentrations.[178] The 20% of women who were nulliparous in the Rotterdam Study may include a large percentage of women who were nulliparous by choice. These 3 studies suggest that women of higher parity and nulliparous women with fertility problems may have greater CCA IMT.

1.5.6 Studies of Arterial Stiffness in Healthy versus Complicated Pregnancies

Many studies have demonstrated an association between greater arterial stiffness and preeclampsia. A 2011 systematic review and meta-analysis included 23 studies of arterial stiffness in women with or after preeclampsia compared to women with healthy pregnancy.[47] The meta-analysis found a greater standard mean difference (SMD) of arterial stiffness measure between women with and without preeclampsia (SMD 1.62, 95% CI 0.73-2.50). This significant increase held for measures of central stiffness: carotid-femoral pulse wave velocity (weighted mean difference (WMD) 1.04, 95% CI 0.34-1.74) and augmentation index (WMD 15.10, 95% CI 5.08-25.11).

This dissertation research includes a measure of peripheral stiffness, brachial artery distensibility. Less research is available on measures of peripheral stiffness and preeclampsia. While peripheral stiffness reflects central stiffness, it is also influenced by different factors that might give insights into different mechanisms of arterial remodeling. In the meta-analysis above, the measure of peripheral stiffness, carotid-radial PWV, was non-significantly higher in women with preeclampsia (WMD 0.99, 95% CI -0.07 – 2.05). Two of three studies[179,180] included in the carotid-radial PWV analysis showed significant increases; the one that did not included only 6 women with preeclampsia.[161] All three of the studies included examined PWV during pregnancy; the one that included a postpartum measure found no significant difference at 7 weeks postpartum.[179] Other studies, summarized in Table 2.5, using peripheral or mixed measures, found stiffer arteries during pregnancy for women with or preceding preeclampsia compared to women with healthy pregnancies[181,182], and immediately postpartum (3-5 days)[181] but not later postpartum,[181,183] although one showed a trend towards higher peripheral PWV velocity in postpartum women after preeclampsia.[183]

1.5.7 Prospective Studies of Arterial Stiffness in Healthy Pregnancy

Early studies demonstrated that the aorta is more compliant in pregnancy, leading to general statements about decreased vascular stiffness in healthy pregnancy,[155] but more recent longitudinal studies suggest that there are changes in stiffness over the course of pregnancy, and that changes may differ between arterial beds. Two studies have shown the brachial-ankle PWV, aortic augmentation index, carotid-femoral PWV and carotid-radial PWV are either stable or drop between the first and second trimesters of pregnancy, then increase in the third trimester and remain elevated postpartum.[179,181] In contrast, another group found that carotid-femoral

PWV dropped throughout pregnancy (indicating decreased stiffness), while the carotid compliance and distensibility both dropped (indicating increased stiffness).[172] The latter study supports earlier work by the same group that also demonstrated carotid stiffening.[171]

1.6 GAPS IN KNOWLEDGE AND GOALS OF DISSERTATION

The theoretical framework for this dissertation is Holly Powell Kennedy's Model of Exemplary Midwifery Practice.[184] This model highlights midwifery's belief in and respect for pregnancy and childbirth as normal processes. Research into these normal processes is respectful of women, contributes to women achieving optimal health, and enhances the profession of midwifery.

The complex interplay between cardiovascular risk factors, acute and long-term hemodynamic, metabolic, and hormonal changes of pregnancy, utero-placental vascular remodeling, systemic vascular remodeling, pregnancy complications, and CVD risk, is represented as a conceptual model in Figure 1.5. Factors such as age, race and BMI influence pre-pregnancy uterine and systemic vasculature. During pregnancy, acute changes in hemodynamics, metabolism and hormone concentrations influence remodeling of both the utero-placental and systemic vasculature; invasion by EVT cells plays a major role in remodeling of the spiral arteries in the decidua. The adequacy of the remodeling of the utero-placental vasculature influences risk of pregnancy complications; remodeling of the systemic vasculature may also affect this risk. Postpartum, both parts of the vasculature change, but it is unclear if they return to baseline levels. The baseline structure of the systemic vasculature, the changes

that happen to it during pregnancy, and the effect of long-term hemodynamic, metabolic and hormonal changes after pregnancy influence remodeling of the vasculature after childbearing, which then affects CVD risk.

Some parts of this model are fairly well understood. There is significant understanding of how CVD risk factors influence systemic vascular remodeling, and how vascular remodeling then is itself a CVD risk factor. Many studies have described the hemodynamic, hormonal and metabolic changes of pregnancy and the remodeling of the utero-placental vasculature. It is understood that inadequate utero-placental vascular remodeling is related to pregnancy complications.

Most parts of the model are not well understood. There is little understanding of what risk factors influence utero-placental vascular remodeling and risk of pregnancy complications. Small cross-sectional studies suggest that systemic vascular remodeling in pregnancy may reflect the degree of utero-placental vascular remodeling, but much more work is needed to understand this relationship. Only very small prospective studies have described systemic vascular remodeling in pregnancy, and these were not done in women reflective of the US population. We do not know the extent to which uterine and systemic vasculature return to baseline in the postpartum period. Only cross-sectional studies explore the relationship between pregnancy and long-term vascular health.

A full understanding of these complex relationships requires a life-course approach far beyond the scope of a doctoral dissertation. It would require large cohort studies following women from childhood to death, with measurement of risk factors and measures of the vasculature at multiple points. It would require participation from health care providers, epidemiologists, and basic scientists.

Nonetheless, this dissertation may help elucidate 3 parts of the model, using data from two different studies. The Slow Adverse Vascular Effects (SAVE) clinical trial provided healthy overweight and obese young adults with an intensive lifestyle intervention aimed at weight loss and explored changes in their vascular measures. The reproductive ancillary study collected reproductive data on women participants in SAVE. Paper 1 explores the relationship between parity and remodeling of the carotid artery in women in SAVE, illuminating how pregnancy changes may persist post-childbearing. The Maternal Adaptation to Healthy Pregnancy Study (MVP) prospectively assessed CCA IMT, IAD, and brachial artery distensibility, in 43 healthy primigravid women throughout pregnancy and postpartum. Paper 2 analyzes the changes that occur in CCA IMT and IAD and Paper 3 the changes in brachial artery distensibility during pregnancy. Both MVP papers address factors that are associated with these structural and functional changes including maternal weight, weight gain, and blood pressure, and glucose, lipid and C - reactive protein concentrations.

1.7 SPECIFIC AIMS

Paper 1:

Specific aim 1: To investigate the relationships between reproductive factors and the prevalence of subclinical cardiovascular disease in women enrolled in the SAVE study.

Hypothesis: Higher parity will be associated with greater intima-media thickness.

Paper 2:

Specific aim 1: To prospectively assess common carotid artery adventitial diameter and intima media thickness throughout healthy first pregnancy and postpartum.

Hypotheses:

- During the course of a normal pregnancy adventitial diameter will increase, followed by increase in intima media thickness. These will return to baseline postpartum.
- Pre-pregnancy body mass index will be related to increases in common carotid artery diameter and intima-media thickness.
- Increase in weight over the course of the pregnancy will be the primary factor associated with increase in common carotid artery diameter and intima media thickness.

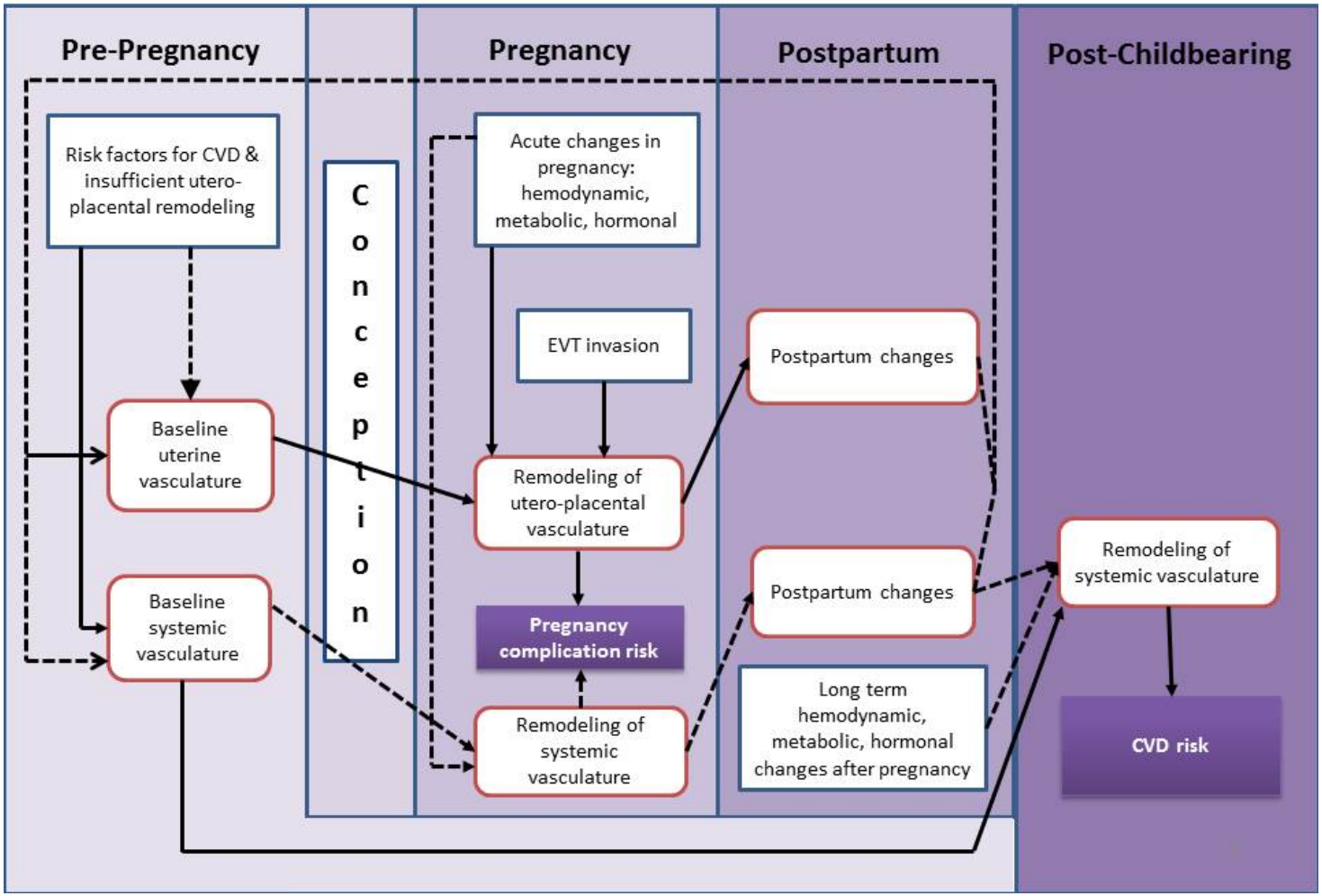
Paper 3:

Specific aim 1: To prospectively assess brachial artery stiffness throughout healthy pregnancy and postpartum.

Hypothesis: The brachial artery will remain elastic throughout the course of pregnancy.

Table 1.1 Hemodynamic and Metabolic Changes of Healthy Pregnancy

Hemodynamic	Increased cardiac output
	Increased plasma volume
	Increased heart rate
	Decreased systemic vascular resistance
	Decreased mean arterial pressure (returns to baseline at term)
Metabolic	Increased insulin resistance
	Lower fasting and higher post-prandial glucose concentrations
	Increased insulin concentrations
	Increased lipid concentrations (total cholesterol, triglycerides, LDL-c, HDL-c)



CVD is cardiovascular disease. EVT is extravillous trophoblast cells. Solid lines represent well-understood pathways. Dashed lines represent less well-understood pathways.

Figure 1.1 Conceptual model relating remodeling of systemic and utero-placental vasculature

2.0 NULLIPARITY IS ASSOCIATED WITH LESS HEALTHY MARKERS OF SUBCLINICAL CARDIOVASCULAR DISEASE IN OVERWEIGHT AND OBESE YOUNG WOMEN

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2.1 ABSTRACT

Objective: Higher parity is associated with m subclinical cardiovascular disease (CVD) in mid-life and older women, and with increased CVD risk overall. The relationship between parity, subclinical CVD, and infertility in overweight and obese young women has been infrequently evaluated.

Methods: Reproductive histories were obtained in 191 (73%) overweight and obese (BMI 25 – 39.9 kg/m^2) young women participating in a weight loss trial. Baseline carotid intima-media thickness (IMT) and inter-adventitial diameter (IAD) were assessed via B-mode ultrasound. Linear regression was used to estimate the relationship between parity and carotid measures, adjusted for demographic, cardiovascular and reproductive risk factors.

Results: Nulliparous women (n=70, age 34.9 ± 7.1) had increased common carotid IAD (.230 mm, SE .08, P = .006) and mean CCA IMT (.031 mm, SE .01, P = .009) compared with parous women (n=102, age 39.5 ± 4.9) after adjustment for age, race, and CVD risk factors. No other reproductive factors were statistically significantly associated.

Conclusions: Nulliparity is associated with markers of less healthy carotid arteries in a sample of disease-free, overweight or obese 25-45 year-old women. This may represent a beneficial effect of pregnancy or indicate overall better health in overweight/obese women capable of childbearing.

2.2 INTRODUCTION

Nearly two-thirds of US women of childbearing age are at elevated risk for cardiovascular disease (CVD) because they are overweight or obese,[185] but little is known about how reproductive factors influence CVD risk in this population. Studies demonstrate a relationship between parity (number of births after 20 weeks gestation)[186] and CVD risk in the general population. A cohort study of more than a million Swedish women demonstrated a J-shaped relationship, with lowest CVD risk in women with 2 births. Women of lower parity had a 10% increased risk, and women of parity 5+ had a 50% increased risk.[77] This population, however, is leaner and more homogeneous than the US population. Understanding the relationship between parity and CVD risk in overweight women may lead to better screening and interventions to decrease excess risk of high or low parity in this already high risk group.

Changes in inter-adventitial diameter (IAD) and intima-media thickness (IMT) of the carotid arteries serve as markers of vascular aging and can be measured reliably and non-invasively using high frequency B-mode ultrasound. Multiple studies have demonstrated that increased IAD and thicker IMT are associated with traditional CVD risk factors and ultimately with cardiovascular events,[26,39] and they are often used as surrogate markers for CVD risk. Several investigations have examined the relationship between parity and IMT with inconsistent results. In a prospective study of women of childbearing age, each birth over a 6 year period was associated with a 7.5 ± 3.2 μm increase in mean IMT of the common carotid artery (CCA).[85] Cross-sectional studies have largely shown increased CCA IMT with increased parity in

midlife[175,176] and younger women;[84] one study also showed increased CCA IMT in nulliparous women.[176]

Thus, increases in CCA IMT with parity may reflect a potential mechanism linking parity to CVD, but many questions remain unanswered. Women in the aforementioned studies were relatively lean and not representative of the current US population. They were primarily mid-life or older. The existing studies did not evaluate the effect of infertility on CVD risk, ignoring a potentially significant confounding factor. In the majority of studies, the CCA, not other segments of the carotid artery, was assessed. IMT measured in different segments of the carotid artery have different CVD risk factor associations and may reflect varying pathophysiology, thus providing further insight into the underlying CVD mechanisms involved.[187] Body mass index is most closely related to CCA IMT, while glucose metabolism is more related to bulb and ICA IMT.[187] Furthermore, none of the studies assessed parity's relationship with IAD, itself an independent CVD risk factor and marker of vascular remodeling and aging.[7,39]

The purpose of this analysis is to explore the relationship between parity and structure of the common and internal carotid arteries and the carotid bulb in a US population of overweight or obese women of reproductive age without history of fertility problems. We hypothesized that parity would be positively related to IMT and IAD in the CCA.

2.3 METHODS

2.3.1 SAVE Study Design and Population

This study is a secondary analysis of data from the Slow Adverse Vascular Effects (SAVE) clinical trial (NCT00366990). Methods for participant recruitment and intervention in

the SAVE trial have been previously reported.[188] Briefly, SAVE is a randomized controlled trial examining effects of weight loss, physical activity, and sodium reduction on vascular health. Participants (N = 349) were 25-45 year-old women and men from Allegheny County, PA, who were physically inactive and overweight to class II obese (BMI 25 – 39.9). Exclusions were: 1) diabetes; 2) hypertension; 3) current use of cholesterol-lowering, antipsychotic, or vasoactive medicines; 4) underlying inflammatory conditions; 5) known atherosclerotic disease; and 6) pregnancy or breastfeeding. No other requirements about reproductive history were included. All participants signed an informed consent document approved by the University of Pittsburgh Institutional Review Board. SAVE enrolled 290 women between June 2007 and February 2009; 191 (66%) completed the reproductive history and 19 reported infertility, leaving 172 (59%) women for the analytic sample for this study.

2.3.2 Carotid Artery Measures

Carotid ultrasounds were performed at the University of Pittsburgh Ultrasound Research Laboratory (Pittsburgh, PA) using high resolution B-mode ultrasound (Siemans, Malvern, PA). This analysis uses the baseline values. The carotid arteries were imaged bilaterally at end diastole with participants supine. IMT, the distance from the media- adventitial interface to the intima-lumen interface, was measured bilaterally in four locations: the near and far walls of the common carotid artery 1 cm proximal to the carotid bulb, the far wall of the carotid bulb, and the far wall of the internal carotid artery (ICA) for the first 1 cm distal to the flow divider. Mean and maximum values were calculated for each carotid segment and the mean and maximum of the eight readings identified. Inter-adventitial diameter was measured as the distance from the adventitial-medial interface of the near wall of the common carotid artery to the medial -

adventitial interface of the far wall. Reproducibility of IMT was excellent with an intraclass correlation coefficient of ≥0.82 between sonographers and ≥ 0.97 between readers.[188] A semi-automated reading program (AMS system) allowed the reading to be done by computer. [189]

2.3.3 Reproductive Histories

Starting June 1, 2009, all women participants were given reproductive history forms to complete. Studies have demonstrated high reliability and validity for maternal recall of pregnancy-related events.[190,191] Participants reporting they had ever been pregnant then completed a form for each pregnancy. They designated each birth outcome as a live birth, tubal/ectopic pregnancy, abortion, miscarriage (fetus born before 20 weeks or 5 months gestation), stillbirth (baby lost after 20 weeks or 5 months gestation) or current pregnancy. Parity was calculated as the sum of live (n=257) and stillbirths (n=6). Women who reported a pregnancy since their baseline visit were coded with their parity at baseline.

Each pregnancy form asked for date of pregnancy outcome, birth weight, length of gestation, presence of specific pregnancy complications, and breastfeeding duration. Women were coded as having had any pregnancy complication if they reported a stillbirth, preeclampsia or gestational hypertension in any pregnancy, or a birth weight of < 2500 grams or gestational age of less than 37 weeks for any singleton pregnancy. Women were designated as having a history of infertility (n=19) if they answered yes to either: “Have you ever had a period of 12 months when you could not get pregnant although you would have liked to get pregnant” or “Have you ever taken any fertility medication to help you get pregnant?” Women reported usual menstrual cycle length and whether they had menstruated in the past 12 months.

2.3.4 Demographic, Physical, and Laboratory Measures

Participants provided self-reported information at baseline regarding age, gender, race, and smoking status. Staff measured height, weight and blood pressure using standardized protocols. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Laboratory assays were performed on fasting serum samples at the Heinz Laboratory at the University of Pittsburgh (Pittsburgh, PA). Total cholesterol, HDL(c), LDL(c), and glucose were determined using standard laboratory procedures.

2.3.5 Statistical Analysis

Normally distributed variables are presented as mean ± SD and categorical variables as percentages. Characteristics of women who did and did not participate in the reproductive study were compared. Due to small numbers of women of high parity (parity 4 n=8, parity 5 n=1), women of parity 3 or greater were analyzed together. As only 5 women reported a race other than white or black, women were classified as black or non-black for the analysis. Because of significant difference in age and racial composition of the different groups, age and race-adjusted means for the carotid measures were calculated. When analysis of variance testing detected a significant difference among these means pair-wise comparisons were done. Inspection of these results demonstrated that IAD and CCA IMT were greatest in nulliparous women and approximately equal in women with parity 1, 2 and 3+ (Figure 1). For this reason we compared nulliparous to parous women for the remaining analyses.

Multivariable linear regression was used to estimate the relationship between parity and the carotid measures. Covariates were considered a priori according to known factors associated with the carotid measures and CVD, and retained in the model if they were statistically significant predictors and affected the parameter estimates for any of the outcomes. The first model was adjusted for age, race, and educational achievement, our chosen measure of socioeconomic status. The second model included adjustment for those demographics plus baseline cardiovascular risk factors: BMI, current smoking, use of alcohol, average systolic blood pressure, fasting glucose and non-HDL cholesterol concentration. Educational achievement and non-HDL cholesterol concentration did not meet inclusion criteria and were dropped from the model to maintain precision. A third model was considered including all of the previous covariates with reproductive factors: menopausal status, regularity of menstrual periods, breastfeeding, and the composite variable for pregnancy complications. None of these met the criteria for inclusion in the final model. Thus, the main model included parity, age, race, BMI, current smoking, alcohol use, systolic blood pressure and fasting glucose level. We explored interactions between BMI category (25-29.9, 30-34.9, 35+) and parity and between race and parity in this model, and also adjusted the IMT models for IAD.

A separate analysis was done for parous women to estimate the effects of levels of parity (1, 2 or 3+) on carotid measures using the main model as with additional factors unique to parous women: age at first birth and time since last birth. These factors were not significant predictors and results are not presented here.

A sensitivity analysis to test for potential residual confounding based on infertility history considered a model including all women in SAVE to test the effect of known infertility on the carotid measures, controlling for parity, demographic and cardiovascular risk factors.

All statistical analyses were performed using SAS 9.2 or 9.3 (SAS Institute, Cary, NC) with significance level set at $p = 0.05$.

2.4 RESULTS

Participants and non-participants in the reproductive study were largely comparable at baseline (Table 2.4). Participants had a lower BMI (32.2 vs. 33.7, $p = 0.01$) and higher total (207.2 vs. 193.9, $p = 0.01$) and LDL (125.2 vs. 116.3, $p = 0.03$) cholesterol. For participants, average age increased with parity, from 34.9 years old for nulliparas to 40.7 for women of parity 3+ (Table 2.1). Black women were over-represented in the group with parity of 1 (46%, vs. $\leq$ 14% in the other groups). Nulliparous women were more likely to regularly consume alcohol. By study design all women were overweight or obese, and mean BMI of 32 did not differ significantly by parity.

Among traditional CVD risk factors, only HDL(c) differed by parity and was highest in nulliparous women. Nulliparous women tended to be current smokers compared to women of parity 3+ (12.9% vs 0.0%). Average systolic blood pressure, fasting glucose, total cholesterol, LDL(c), and triglycerides were similar among all groups.

For reproductive factors, history of irregular menstrual periods was similar in each group. Among parous women, age at first birth and rate of reporting any pregnancy complication were similar. Women of higher parity were more likely to have ever breastfed and to be post-menopausal, and had a shorter time since their last birth.

Nulliparous women had the highest values for IAD, average CCA IMT, maximum CCA IMT, average IMT and maximum IMT (Table 2.2). For the 3 measures in the CCA, nulliparous

women had the highest values and values for the 3 levels of parity are roughly equivalent. When comparing the adjusted means for nulliparous vs. parous women these results were statistically significant (Figure 2.1). Patterns were less obvious for the other carotid measures. There was no clear pattern for internal carotid artery IMT. Bulb IMT, however, was significantly greater for nulliparous women and for women with 3 or more births than for women with 2 births.

Relationship between having ever given birth and the carotid measures is presented in Table 2.3. After adjustment for demographics and cardiovascular risk factors nulliparous women had IAD 0.20 mm larger (p=0.006), average CCA IMT 0.03 mm thicker (p=0.010) and maximum CCA IMT 0.04 mm thicker (p= 0 .004) than parous women. Average bulb IMT, average IMT, and maximum IMT were also greater in nulliparous women, but these differences do not reach significance. There were no differences in average ICA IMT based on parity. Adjustment for IAD attenuated the differences in IMT.

There was no significant interaction between BMI and nulliparity. In the final model the interaction term for black race and parity was statistically significant for mean CCA IMT (p=0.01) and borderline significant for maximum CCA IMT (p=0.058). Figure 2.2 shows that black women had thicker mean and maximum CCA IMT than did non-black women, and the difference between IMT for nulliparous and parous women appeared greater for blacks than for non-blacks.

In the sensitivity analysis including the 19 women with a known infertility history, infertility was not a significant predictor of any carotid outcome, although the effect size was similar to the effect size of nulliparity for mean and maximum CCA IMT (Table 2.5).

2.5 DISCUSSION

This analysis demonstrates that in a sample of disease-free overweight and obese young women without known infertility history, nulliparity is associated with less healthy markers of subclinical CVD even after accounting for traditional CVD risk factors. Nulliparous women had greater IAD and mean and maximum CCA IMT compared to parous women, and thicker bulb IMT compared to women with 2 births. Increased IAD seemed to mediate some, but not all of the difference in IMT measures. This might describe a first birth effect – a one-time change occurring after first birth but not repeated after subsequent births - as seen with decreased HDL-c and systolic blood pressure after a first birth.[65,66] Or it may represent the left peak of a J-shaped relationship, similar to the J-shaped relationship seen between parity and cardiovascular risk. The small number of women of higher parity makes it difficult to discern if CCA IMT or IAD might increase with higher numbers of births.

Our results contradict findings of some studies. In the Rotterdam Study, among women aged 55-99 years old, there was a trend towards increased mean and maximum CCA IMT with increased parity, significant even after adjustment for demographics and cardiovascular risk factors.[175] The Study of Health in Pomerania detected a U-shaped association between CCA IMT and parity in women aged 45-79 years old. After adjustment for demographic, cardiovascular risk, and reproductive factors, greatest mean and maximum CCA IMT were in women with parity of 0 or 3 or greater.[176] Different rates of nulliparity (20% in the Rotterdam Study and 8.5% in the Study of Health in Pomerania) may explain the contradiction. The authors of the Pomerania study suggest that in the Rotterdam Study many women were nulliparous by

choice, while in their population most nulliparous women had true infertility. Women with infertility may be at higher risk for CVD because of hormonal conditions such as polycystic ovary syndrome that can lead to both infertility and increased CVD risk factors.[79] Our study addressed this issue by removing women with known infertility from the analysis.

These studies both looked at mid-life and older women, as opposed to the younger women in our study. It is possible that pregnancy has differing effects on vasculature at different times. Our study may show short term positive effects on vasculature from the increased hormone levels and cardiac output of pregnancy, while studies in older women may reflect negative long-term effects of pregnancy on lipid levels, glucose metabolism, and weight and body fat. Women in these studies were also leaner than women in our analysis, with average BMI's of about 28 and 27, compared to the average BMI of 32 in our study. This suggests that the effects of pregnancy on the vasculature might differ in women who are and are not obese.

Our results also differ from those of the only study done in women of childbearing age. The Cardiovascular Risk in Young Finns Study followed 1005 women aged 24-39 years old for 6 years and found that mean CCA IMT increased 0.0075 mm for each birth occurring during that time period, after adjusting for demographic and cardiovascular risk factors.[85] The authors speculated this increase may be a result of atherogenic metabolic changes in pregnancy. The women in the studies differ substantially; women in the Young Finns study were substantially leaner, with average BMI of about 24. It is possible that pregnancy has different net effects on vascular health depending on women's risk factor profiles, including BMI.[84]

Our finding that carotid bulb IMT is not associated with nulliparity is consistent with the findings of Kharazmi et al. They studied 746 Finnish women between the ages of 45 and 74 years old and found no relationship between parity and the mean of the IMT in the CCA and the

carotid bulb, after adjustment for demographic and cardiovascular risk factors.[177] We found different patterns of change in the CCA and bulb, suggesting they should not be analyzed together. This is supported by various population-based studies that found that bulb IMT is related to true atherosclerosis, while CCA IMT is more a marker of vascular aging and adaptation.[187]

One possible explanation for increased CCA IMT and inter-adventitial diameter in nulliparous women is potential residual confounding from fertility status. While we removed all women reporting infertility from the sample, some nulliparous women may have never attempted pregnancy and could have unidentified fertility problems. If these women had greater IMT and IAD, the increased values we found in nulliparous women may be from the influence of unidentified infertility, not nulliparity. In our model comparing women with and without known fertility problems the effect size of infertility was very similar to the effect size of nulliparity for mean and maximum CCA IMT. This suggests that some of the nulliparous women may have unknown infertility, and that infertility-related problems may be the cause of the less healthy arterial parameters.

Another possible explanation is that these results represent a beneficial effect of pregnancy on the vasculature. Several studies of midlife women demonstrate that higher levels of estrogen are associated with lower IMT and/IAD.[100,101,105,106] Perhaps we are seeing a lasting positive effect from increased pregnancy hormones assessed an average of 9 years after pregnancy.

Two findings relating to health equity deserve further study. Lower educational attainment was associated with thicker average and maximum IMT after adjustment for age and race, but not after adjustment for CVD risk factors. This suggests that CVD risk factors may

mediate a relationship between less education and CVD, and merits thorough exploration in a larger study with a more diverse population. In our study, as in others, [26,192,193] black women had worse measures of subclinical CVD than non-black women; our study adds that differences between nulliparous and parous women were greater for black women, and indeed, may only be present in black women. This suggests that overweight, black, nulliparous women might represent a group at particularly elevated risk for CVD, and should be explored further.

This analysis is one of the only studies of the relationship between parity and vascular health in overweight and obese young women and it uses well-validated, reliable measures of IMT and inter-adventitial diameter. Limitations include small sample size and reliance on self-report pregnancy histories. Lack of healthy weight participants makes aspects of the results difficult to interpret; a life course epidemiologic study would be necessary to determine the full trajectory of the relationship between reproductive events and changes in adiposity and markers of vascular remodeling. The consideration of multiple IMT outcomes may increase the risk of Type 1 error, and P-values should be considered in this light. The study selection criteria are both a strength and a limitation. By including only overweight or obese women who are non-diabetic and normotensive the study limits the generalizability of the results and also limits its ability to determine fully whether pregnancy's effects on BMI, glucose metabolism, and blood pressure mediate pregnancy's effect on the vasculature. At the same time, by focusing on a uniform group of women, the study is able to limit confounding factors and focus on the effects of the pregnancy itself.

Pregnancy affects cardiovascular health in many ways and the ability to achieve successful pregnancy is a marker of good health. This study demonstrates that nulliparous overweight and obese young women show poorer vascular health compared to parous women, as

measured by greater CCA IMT and inter-adventitial diameter. Nulliparous overweight and obese women, particularly black women, may be at increased risk for CVD even if they are normotensive and non-diabetic, and may particularly benefit from risk reduction efforts.

2.6 TABLES AND FIGURES

Table 2.1 Baseline characteristics of participants in SAVE reproductive substudy by parity

	Nulliparous n = 70	Parity 1 n = 26	Parity 2 n = 45	Parity 3+ n=31	Overall P Value	Nulliparous vs. Parous P Value
Age (yr)	34.9 ± 7.1	37.5 ± 6.1	39.8 ± 4.6	40.7 ± 3.8	0.000	<.000
Black race	10 (14.3)	12 (46.2)	7 (15.6)	5 (16.1)	0.004	0.135
College graduate	58 (82.9)	14 (53.9)	28 (66.6)	18 (58.0)	0.162	0.014
Body mass index (kg/m^2)	32.5 ± 4.5	32.3 ± 4.1	32.1 ± 3.4	31.8 ± 3.1	0.889	0.550
Alcohol consumption > 1/month	41 (58.6)	9 (34.6)	14 (31.1)	14 (45.2)	0.020	0.004
Average systolic blood pressure	112.4 ± 9.8	112.6 ± 8.8	112.2 ± 9.9	112.3 ± 9.9	0.999	0.959
Current smoking	9 (12.9)	3 (11.5)	3 (6.7)	0 (0.0)	0.174	0.112
Fasting glucose (mg/dl)	97.1 ± 8.7	97.2 ± 8.1	97.2 ± 8.6	97.6 ± 7.3	0.995	0.863
Total cholesterol (mg/dl)	205.3 ± 38.9	207.1 ± 38.5	198.6 ± 35.7	213.1 ± 42.8	0.452	0.977
LDL (mg/dl)	118.1 ± 35.8	129.3 ± 36.4	120.9 ± 28.1	133.8 ± 37.4	0.142	0.098
Triglycerides (mg/dl)	138.8 ± 76.8	116.4 ± 134.6	113.0 ± 60.0	128.7 ± 53.3	0.353	0.110
HDL (mg/dl)	60.0 ± 15.2	55.1 ± 14.9	55.1 ± 11.4	53.7 ± 11.5	0.091	0.012
Reproductive History						
Irregular menses	5 (7.1)	0 (0.0)	2 (4.4)	2 (6.5)	0.552	0.351
Post-menopause	4 (5.7)	3 (11.5)	2 (4.4)	7 (22.6)	0.030	0.180
Any pregnancy complication	-	8 (30.8)	14 (31.1)	13 (41.9)	0.563	
Ever breastfed	-	16 (61.5)	39 (86.7)	26 (83.9)	0.032	
Age at first birth (yr)	-	25.5 ± 5.9	26.2 ± 4.7	24.7 ± 4.5	0.457	
Time since last birth (yr)	-	11.6 ± 6.9	9.0 ± 6.1	7.7 ± 3.6	0.040	

Values shown are means ± SDs or frequency counts (with percentages).
P values from analysis of variance or chi-square test, as appropriate.

Table 2.2 Age and race-adjusted mean carotid measures by parity in women without infertility history

	Nulliparous n = 70	Parity 1 n = 26	Parity 2 n = 45	Parity 3+ n = 31	P value
Carotid Measure (mm)					
Inter-adventitial Diameter	6.942 (.057)	6.794 (.093)	6.657 (.069)*	6.722 (.086)†	0.018
Average CCA IMT	.610 (.008)	.579 (.013)	.579 (.010)	.577 (.012)	0.062
Maximum CCA IMT	.719 (.010)	.682 (.016)	.686 (.012)*	.671 (.015)*	0.037
Average ICA IMT	.547 (.016)	.575 (.027)	.518 (.020)	.567 (.024)	0.254
Average bulb IMT	.688 (.016)	.672 (.025)	.627 (.019)†	.713 (.023)‡	0.018
Average IMT	.613 (.009)	.601 (.015)	.576 (.011)	.606 (.014)	0.084
Maximum IMT	.770 (.013)	.748 (.021)	.724 (.016)	.756 (.019)	0.167

Values shown are means (standard error).
* Different from nulliparous at p <.01
† Different from nulliparous at p <.05
‡ Different from parity 2 at p <.01
P values from analysis of variance.

Table 2.3 Carotid measures for parous vs. nulliparous women. Regression coefficients represent change from a baseline parity of 0

	Age & Race adjusted		Model 1[1]		Model 2[2]	
Carotid Measure (mm)	**ß (SE)**	**P value**	**ß (SE)**	**P value**	**ß (SE)**	**P value**
Inter-adventitial Diameter	.230 (.077)	.0032	.201 (.073)	.0064	-	-
Average CCA IMT	-.031 (.011)	.0067	-.028 (.011)	.0095	-.017 (.010)	.0948
Maximum CCA IMT	-.039 (.014)	.0050	-.038 (.013)	.0036	-.028 (.013)	.0298
Average ICA IMT	.000 (.022)	.9889	.003 (.022)	.9013	.006 (.022)	.7791
Average bulb IMT	-.024 (.021)	.2589	-.013 (.021)	.5351	-.009 (.021)	.6769
Average IMT	.022 (.017)	.0801	-.017 (.011)	.1422	-.009 (.012)	.4292
Maximum IMT	-.030 (.017)	.0811	-.025 (.016)	.1299	-.018 (.017)	.2901

[1]Model 1 represents the simultaneous effects of parity, age, race, BMI, current smoking, use of alcohol, average systolic blood pressure, and fasting glucose level on the carotid measures.
[2]Model 1 with inter-adventitial diameter.
P values represent significance of regression coefficient for parity.

Table 2.4 Comparison of participants and non-participants in reproductive study

Characteristic	Non-participant n=79	Participant n=191	P value
Age (yr)	37.9 ± 6.1	38.0 ± 6.2	0.95
Black	16 (20.3)	35 (18.3)	0.71
College graduate	64 (81.0)	134 (70.2)	0.07
Body mass index (kg/m^2)	33.7 ± 3.7	32.2 ± 3.9	0.01
Alcohol consumption > 1/month	31 (39.2)	87 (45.6)	0.34
Average systolic blood pressure	112.5 ± 12.2	112.4 ± 9.9	0.95
Current smoking	7 (8.9)	7 (8.9)	0.99
Fasting glucose (mg/dl)	97.4 ± 8.1	97.1 ± 8.3	0.81
Total cholesterol (mg/dl)	193.9 ± 33.9	207.2 ± 39.4	0.01
LDL (mg/dl)	116.3 ± 28.7	125.2 ± 35.7	0.03
Triglycerides (mg/dl)	118.6 ± 64.4	129.8 ± 81.4	0.23
HDL (mg/dl)	54.0 ± 11.4	56.4 ± 14.0	0.14

Values shown are means ± SD or frequency counts (with percentages).
P values from analysis of variance or chi-square test, as appropriate.

Table 2.5 Effect of infertility on common carotid artery measures

Carotid Measure (mm)	ß (SE)	P value
Inter-adventitial diameter	.047 (.11)	0.658
Average CCA IMT	.027 (.02)	0.092
Maximum CCA IMT	.024 (.02)	0.201
Average ICA IMT	.023 (.03)	0.462
Average bulb IMT	-.005 (.03)	0.872
Average IMT	.018 (.02)	0.287
Maximum IMT	.015 (.02)	0.522

Model represents the simultaneous effects of parity, age, race, BMI, smoking status, use of alcohol, average systolic blood pressure and fasting glucose level on the carotid measure. P value is for regression coefficient for infertility.

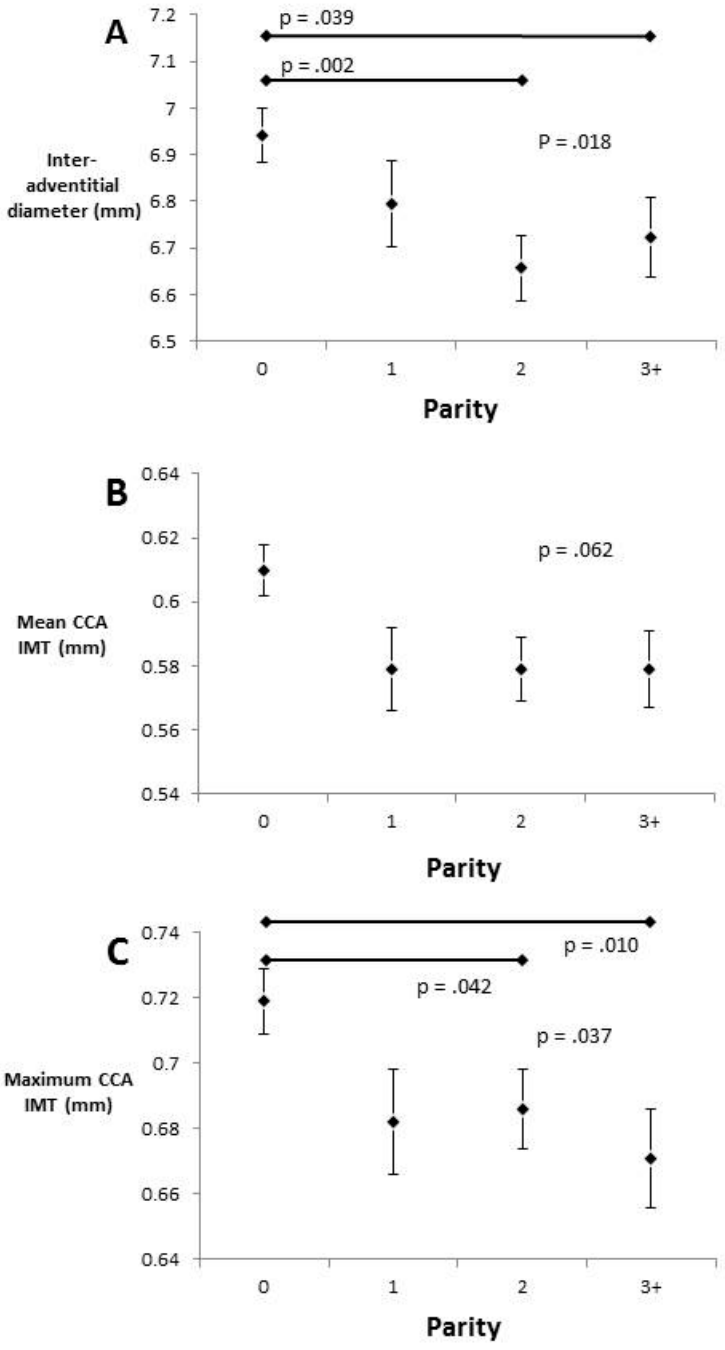

Figure 2.1 Age and race-adjusted carotid artery measures by parity

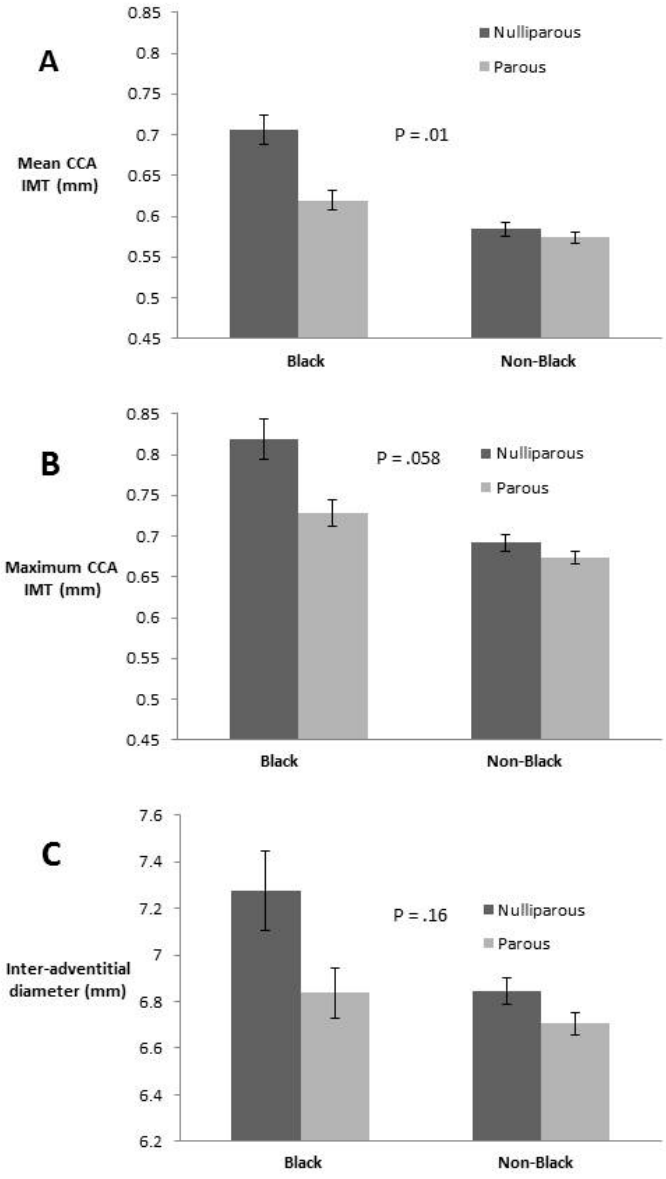

Figure 2.2 Interaction between race and parity, adjusting for age, body mass index, smoking, alcohol use, systolic blood pressure and fasting glucose levels. P values are for race x parity interaction

3.0 COMMON CAROTID ARTERY INTIMA-MEDIA THICKNESS INCREASES OVER PREGNANCY CYCLE

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3.1. ABSTRACT

Objectives: Higher parity is associated with greater subclinical cardiovascular disease (CVD) in mid-life and older women, and with higher risk of CVD overall. Prospective studies of arterial change throughout normal pregnancy are lacking; without them it is unclear whether unhealthy changes of the vasculature during pregnancy persist postpartum and raise women's risk of CVD. The goal of this study was to prospectively assess normal vascular adaptation in healthy pregnant women. Our hypotheses were that during the course of healthy pregnancy: 1. CCA inter-adventitial diameter (IAD) will increase, then return to baseline postpartum, and 2. CCA IMT will initially thin, then thicken as pregnancy progresses, and return to baseline postpartum.

Methods: The Maternal Vascular Adaptations to Healthy Pregnancy Study (Pittsburgh, PA, 2010-2013) assessed 43 healthy women during each trimester of their first pregnancy and 6-8 weeks postpartum at which time points B-mode ultrasound imaging measures of CCA IMT and IAD, independent predictors of CVD risk, were obtained. Linear mixed models were used to compare measures of CCA IMT and inter-adventitial diameter at each time point, after adjustment for age and pre-pregnancy body mass index. Various physical and cardiometabolic measures were then considered as mediators of the relationship between pregnancy and carotid measures.

Results: There were 37 women (age 28.2 ± 4.5 years, pre-pregnant BMI 24.4 ± 3.2 kg/m^2) with uncomplicated pregnancies. After adjustment for age and pre-pregnancy BMI, mean (SE) CCA IAD (mm) increased each trimester, from 6.38(0.08) in the 1st trimester to 6.92(0.09) in the 3rd

trimester, and returned to first trimester levels, 6.35 (0.07), postpartum. Mean (SE) CCA IMT (mm) was increased postpartum (0.567 (0.01)) compared to 1st (0.539 (0.01)) and 2nd trimester values (0.546 (0.01), $p < .05$ for each). In multivariable models, higher systolic blood pressure and lower insulin resistance and C-reactive protein were associated with greater CCA IMT, but did not explain the change occurring during the pregnancy cycle.

Conclusions: As hypothesized, in uncomplicated first pregnancies CCA IAD increased throughout and returned to first trimester levels postpartum. However, contrary to our hypotheses, CCA IMT was increased postpartum. In uncomplicated first pregnancies, some vascular changes resolved (IAD) and others persisted (CCA IMT). Atherogenic metabolic changes of pregnancy did not fully explain the increase in CCA IMT. Whether this indicates that persistence of specific vascular effects of pregnancy may inform long term CVD risk remains to be explored.

3.2 INTRODUCTION

Higher parity (number of births) is related to higher cardiovascular disease (CVD) risk in women, but the reason for this increase in unknown.[75-78] One posited explanation is that the increased weight and atherogenic metabolic changes of pregnancy create unhealthy structural changes in the systemic vasculature that persist long term.[85,174] Studies that might illuminate this relationship have been limited by inadequate sample size to detect significant differences in vessel parameters,[171,194] lack of collection of serial measures of vessel parameters throughout

pregnancy,[85] use of non-standard techniques to assess the vasculature,[173,174] and lack of biomarker collection throughout pregnancy.[85,171,173,174,194]

B-mode ultrasonography of the carotid artery, a non-invasive, reproducible technique to assess arterial structure,[17] can be used to assess structural arterial changes (remodeling) during pregnancy. Both greater intima-media thickness (IMT) and inter-adventitial diameter (IAD) of the common carotid artery (CCA) are associated with higher cardiovascular disease risk factor burden,[25-27] arterial aging,[21] and higher incidence of cardiovascular disease.[26,39] The normal changes that occur in the CCA IMT and IAD during a healthy pregnancy have not been well-established.

The primary objective of this study was to evaluate prospectively the normal course of CCA remodeling measured three times during an uncomplicated first pregnancy and the immediate postpartum period (6-8 weeks). This will illuminate whether normal pregnancies result in unhealthy vascular structural change. A secondary objective was to explore the relationship between pre-conception and pregnancy-related physical and metabolic factors and arterial adaptation. We hypothesized: 1) that IAD would increase early in pregnancy to accommodate increased blood flow and continue greater through the duration of pregnancy; 2) that IMT would initially thin, followed by a thickening later in pregnancy; 3) that pre-pregnancy body mass index and increase in weight will be related to increases in IAD and IMT; and 4) that the higher concentrations of lipids, insulin resistance, and c-reactive protein known to occur in pregnancy would help explain pregnancy's effects on the vasculature.

3.3 METHODS

3.3.1 MVP Study Design and Population

The Maternal Vascular Adaptation to Healthy Pregnancy (MVP) study prospectively assessed common carotid artery parameters in a cohort of healthy women experiencing their first pregnancies. Recruitment consisted of mailings and flyers distributed to the University of Pittsburgh community, use of university research registries, and advertisements in the offices of local (Pittsburgh, PA) prenatal care providers and on the internet. Eligible participants were healthy non-smoking women, age 14–40 years, at less than 38 weeks gestational age in their first pregnancies. Exclusion criteria were: 1) vasoactive medication use; 2) infertility history, defined as ever having had a period of at least 12 months of inability to achieve pregnancy, or using fertility medications to achieve pregnancy; 3) family history of premature coronary artery disease; 4) previous abortion; and 5) multiple gestation. All participants signed an informed consent document approved by the University of Pittsburgh Institutional Review Board.

Study visits were scheduled at 12-14 weeks, 24-26 and 36-38 weeks of pregnancy, and then at 6-8 week postpartum. After a telephone screening for eligibility, women could begin the study at any one of the pregnancy visits. Each visit included physical measures performed by research staff and ultrasound measures of carotid parameters. Visits were conducted between June 2010 and March 2013. The recruitment goal was 46 women; this was based on a calculation that 31 women were needed in order to have 80% power to detect a 0.5 SD difference for change CCA IMT and IAD given an assumed 0.5 correlation among the repeated

observations. It was assumed that 10-20% of women would develop a pregnancy complication and that there would be 25% attrition from the study. Thus, 46 women were targeted in order to obtain 31 women with healthy pregnancies who completed the study. The study enrolled 44 women, of whom 43 had multiple visits and 6 developed pregnancy complications, leaving 37 women in the analytic sample for this analysis.

3.3.2 Carotid Artery Measures

Carotid ultrasounds were performed by the same 2 research sonographers from the University of Pittsburgh Ultrasound Research Laboratory (URL) either at the URL or at the Magee-Womens Hospital Clinical and Translational Research Center. Participants were placed supine, with a right hip wedge for comfort if necessary, and the common carotid artery was visualized at end diastole with high-resolution B-mode ultrasound (ACUSON Cypress System.) The common carotid artery was visualized bilaterally for 1 cm proximal to the carotid bulb, and IMT measured as the distance from the media-adventitial interface to the intima-lumen interface. Approximately 140 images were digitally recorded for each location. IMT reported represents the mean value for near and far wall bilaterally. IAD was measured as distance from the adventitial-medial interface of the near wall to the media-adventitial interface of the far wall. Reading of images was done by computer by one reader, using a semi-automated reading program (AMS) system.[189] Reproducibility of carotid measures at the URL was excellent during the time period of the study, with an intraclass correlation coefficient within the reader of > 0.91 for CCA IMT and >0.99 for IAD.

3.3.3 Demographic, Pregnancy History, Physical and Laboratory Measures

At the initial visit women completed a self-administered demographic form with information on age, race/ethnicity, marital status, education, employment, income, insurance status and intended birth site. Height was measured without shoes using a stadiometer, and the mean of 2 readings from the first visit used. Research staff weighed lightly clothed participants on a standard balance scale, and the mean of 2 readings used. Pre-pregnancy weight was identified preferentially as the pre-pregnancy weight documented in the prenatal record or, if not available, as a documented weight in the medical record in the three months prior to the last menstrual period. Pre-pregnancy body mass index (BMI) was calculated as pre-pregnancy weight in kilograms divided by height in meters squared. Weight change was calculated as the difference between current and pre-pregnancy weight. Prenatal and birth records were reviewed after the final study visit to confirm normalcy of pregnancy and birth and to obtain birth outcome information for future analysis.

After participants rested for 5 minutes in a quiet room, research staff palpated the right radial pulse for 30 seconds and then measured blood pressure in that arm using a mercury sphygmomanometer according to a standardized protocol. Three measurements of each were taken, and the mean of the last 2 used for the analysis. Data on demographic and physical measures and from records reviews were collected and managed using REDCap electronic data capture tools hosted at the University of Pittsburgh.[195] REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data

manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Laboratory assays were performed on fasting serum samples at the Heinz Nutrition Laboratory at the University of Pittsburgh Graduate School of Public Health (Pittsburgh, PA). Total cholesterol,[196] high density lipoprotein (HDL-c),[197] low density lipoprotein (LDL-c)[198], triglycerides[199] and glucose[200] were determined using standard laboratory procedures. Insulin was measured using standard radio-immune assay (Linco Research, St. Charles, MO). HOMA-IR, a measure of insulin resistance, was calculated as (glucose x insulin)/405. [201] High-sensitivity C-reactive protein (hsCRP) was measured with an enzyme-linked immunoassay (Alpha Diagnostics International, Inc. San Antonio, TX).

3.3.4 Statistical Analysis

Distributions were assessed for all variables. Measures with approximately normal distributions were evaluated as means ± standard deviations, measures not normally-distributed (hsCRP and HOMA-IR) were analyzed as medians with interquartile range and log-transformed to meet model assumptions for the analysis, and categorical variables were presented as percentages. Values for measures that were collected over time (CCA IMT, IAD, physical, and laboratory measures) were presented for each of the 4 time points in the study. Linear mixed models with random intercepts and Toeplitz variance and covariance structure were used to estimate means for CCA IMT and IAD, adjusted for maternal age and pre-pregnancy BMI, each trimester and postpartum; to determine the overall effect of time in the pregnancy cycle on these carotid measures; and to determine which specific time points (trimester, postpartum) varied

from each other. The mixed models accounted for differing numbers of study visits by participants.

To determine associations between physical and carotid measures, separate models were constructed including systolic blood pressure, weight, and weight change individually with the effect of time. Maternal age and pre-pregnancy BMI were a priori included in all models. Predictors with a significance level of $P \leq 0.2$ were then placed into models together, and predictors with a significance level of $P \leq 0.1$ were retained. Next, to assess our fourth aim, biomarkers were tested individually in the best models identified for each outcome. These were also considered potential mediators. Biomarkers with a significance level of $P \leq 0.1$ were then placed into the best models together, and significant values retained. A sensitivity analysis was performed eliminating 3 extreme outlier values for hsCRP (≥60 mg/L). To assess whether carotid measures changed over the course of the postpartum period, simple linear regression was performed using weeks postpartum as a continuous predictor of CCA IMT and IAD. P values ≤ 0.05 were considered statistically significant results for the analysis. As a sensitivity analysis, the analysis was repeated using only data from the 15 women who completed all 4 visits.

All statistical analyses were performed using SAS statistical software release 9.3 (SAS Institute, Cary, NC).

3.4 RESULTS

The mean number of study visits was 3.3 (range 2-4), with 15 women completing all 4 visits. At baseline their average age was 28.4 ± 4.6 years and average pre-pregnancy BMI was

24.3 ± 3.3. They were predominantly white (91.9%), married or living as married (89.2%), well-educated (89.1% college graduate or greater) and employed (64.9% full time, 24.3% part time).

Inter-adventitial diameter increased throughout pregnancy from a mean (SE) of 6.47 (.12) mm in the first trimester to 6.89 (.10) mm in the third trimester (all $p< 0.05$), then returned to early pregnancy measurement by the postpartum visit (6.36 (.07) mm, $p= 0.76$) (Table 3.1). Adjustment for maternal age and pre-pregnancy BMI minimally affected these estimates (Figure 3.1). CCA IMT was stable between the first and second trimesters then increased through postpartum (first trimester mean (SE) 0.547 (.02) mm, postpartum 0.565 (.01) mm, $p= 0.018$). These values changed minimally with adjustment for maternal age and pre-pregnancy BMI (Figure 3.2).

Changes in weight, blood pressure, heart rate, cardiac output, and lipid, glucose and hsCRP concentrations followed expected patterns for healthy pregnancies (Table 3.1). We explored whether weight gain, metabolic and inflammatory markers may explain changes seen in IAD and CCA IMT. Greater weight during pregnancy and postpartum was marginally associated with greater IAD and attenuated the increase in IAD that occurred throughout pregnancy (Table 3.4). When metabolic factors were considered as well, higher triglyceride concentrations were significantly associated with lower IAD, and higher hsCRP was significantly associated with greater IAD (Table 3.2). Weight was no longer significantly associated with IAD. When postpartum IAD was examined alone, the amount of time passed since birth did not significantly predict IAD (Table 3.8). At the postpartum visit, 88% of women were fully breastfeeding their infants.

Higher SBP was significantly associated with greater IMT, but accounting for SBP did not attenuate the postpartum increase in IMT (Table 3.3). Current weight and pre-pregnancy

BMI were not significantly associated with IMT (Table 3.5). When metabolic factors were considered as well, greater HOMA-IR was associated with lower IMT values (Table 3.7). Accounting for HOMA-IR did not affect the increased IMT observed postpartum (Table3.3). When postpartum IMT was examined alone, time passed since birth did not significantly predict IMT (Table 3.8).

All study results were consistent with those from the sensitivity analysis including only the 15 women who completed all 4 study visits (data not shown).

3.5 DISCUSSION

This study evaluated the normal course of common carotid artery remodeling that occurs during a healthy first pregnancy and determined that, in agreement with our hypothesis, inter-adventitial diameter of the common carotid artery increased throughout healthy first pregnancy and decreased to baseline postpartum. CCA IMT thickened late in pregnancy and remained thickened into the early postpartum period. Postpartum CCA IMT was greater than the 1st and 2nd trimester values, and no initial thinning of CCA IMT was observed. This was contrary to our hypothesis that IMT would initially thin, then increase. Our results resemble the patterns described in the 2 studies of the topic to date,[171,194] and with more participants than those 2 studies combined our study establishes the statistical significance of those changes.

These basic patterns agree with what is known about hemodynamic changes in pregnancy and the effect of hemodynamic changes on arteries. Arterial walls attempt to maintain homeostasis between the two main stresses from blood flow: shear and tensile stress. Shear stress is the tangential or frictional force of blood flowing along the intima of the artery. The

adaptive response to shear stress is for the diameter of the blood vessel to increase.[7,8,86] Cardiac output increases early in the first trimester of pregnancy[88] peaking at 30-60% above the non-pregnant level in the late 2nd or early 3rd trimester.[88] It would be expected that, as we demonstrated, IAD would increase as cardiac output increases. Tensile stress is the force of blood perpendicular to the wall of the artery, and increases as arterial diameter increases. The adaptive response to tensile stress is for the wall thickness to increase.[7,87] Thus, as we found, it would be expected that IMT would increase during pregnancy as IAD increases, to normalize stresses on the arterial wall.[23]

Our exploratory analysis suggests that neither baseline characteristics of women nor changes in weight fully explained the changes in IAD and CCA IMT that occurred with pregnancy. Triglyceride and hsCRP levels explained some of the changes in IAD, and blood pressure and insulin resistance explained some of the changes in IMT. Neither maternal age nor pre-pregnancy BMI was associated with IAD; older maternal age was not associated with thicker CCA IMT when considered with blood pressure and weight. The increased weight of pregnancy was marginally associated with the increase that occurs in IAD, and may have served as a surrogate for the increased blood volume. As in non-pregnant adults,[24] greater systolic blood pressure was associated with thicker CCA IMT. This represents an adaptive response to the greater tensile stress on the artery.

While our study found metabolic changes in pregnancy that may generally be considered atherogenic in non-pregnant adults (increased total cholesterol, LDL-c, triglycerides, HOMA-IR and hsCRP), these changes did not fully explain the increased IAD and CCA IMT that occurred during the pregnancy cycle, and normally occur during healthy pregnancies. As might be expected, higher hsCRP was associated that with greater IAD. Higher hsCRP concentrations are

associated with greater carotid IMT,[202-204] and thus may be associated with the related IAD as well. Findings that higher triglyceride concentrations were associated with smaller IAD[27] and that greater insulin resistance was associated with thinner IMT[205] were unexpected, as these are different relationships than those found in non-pregnant adults. Paradigms of CVD prediction may not be applicable to the wellness state of pregnancy because of the relatively short timeframe of pregnancy and the dramatic changes in weight, blood volume, and hormonal milieu that occur.

Our finding that CCA IMT was increased postpartum could potentially help explain the increase in CVD risk that occurs in women of higher parity.[75,77] Greater IMT is a risk factor for CVD because thickened arteries are less capable of responding effectively to changes in blood pressure[23] and are more prone to the development of atherosclerosis.[13] Several studies have identified greater IMT in women of higher parity,[84,85,175,176] but it is unknown if the increase is caused by persistence of acute changes of pregnancy or by accumulation of CVD risk factors that occurs with more pregnancies, or is explained by socio-economic differences between women of higher and lower parity. Our study suggests that an acute pregnancy change, thickened CCA IMT, may contribute to higher CVD risk, but that atherogenic metabolic changes in pregnancy do not appear to be the cause.

Potentially, findings from our study may provide important baseline information for future research about pregnancy complications such as preeclampsia. Preeclampsia is a pregnancy-specific disease defined by new origin hypertension and proteinuria occurring after 20 weeks of pregnancy. It complicates 2-8% of pregnancies[55] and contributes to 12% of growth restricted and up to 20% of preterm infants as well as to 50,000 maternal deaths annually.[57] Cross-sectional studies have found greater CCA IMT in women with pregnancies complicated

preeclampsia than in women with normal pregnancies both at term[162] and postpartum.[156,157] Our study provides baseline data that can be used by future prospective studies to help determine when arterial change in pregnancies suffering from preeclampsia deviates from the normal pattern. This may lead to better understanding of the pathophysiology of the disease as well as possible means for early prediction.

This study benefits from use of a highly valid and reproducible measure of carotid structure and from high retention of participants. Although we had serial measure during and after pregnancy, a key limitation of the study is the lack of pre-pregnancy measures. The first visit was scheduled at 12-14 weeks of pregnancy. Significant hemodynamic changes of pregnancy begin as early as 5 weeks of gestation[88] so it is likely that our first trimester values do not represent a true pre-pregnancy baseline. Thinning of the IMT may have occurred before we were able to assess it. The postpartum visit was performed at an average of 8 weeks postpartum. It is likely that not all of the hemodynamic changes of pregnancy have resolved at that point[90] and longer follow-up is required to determine if the increased CCA IMT persists. Participants were overwhelmingly breastfeeding at that point, and the results might not reflect those in women formula feeding their infants. The largely white, well-educated women in this cohort may not be representative of the general population of first time pregnant women, yet the study has utility as providing baseline data against which arterial remodeling in other demographic groups can be assessed. The power calculations for the study were based on the assumption that women would attend all 4 study visits; in actuality, women attended an average of 3.3 visits, decreasing the power of the study. However, the correlation between repeated measures was higher than that posited, and the difference in IAD was greater, providing sufficient power to detect significant differences. The sensitivity analysis including only the 15 women completing

all visits replicated all findings, confirming that failure to attend all the study visits did not negatively affect the results.

We found that IAD increases throughout healthy first pregnancy and decreases by 8 weeks postpartum. In contrast, postpartum CCA IMT is greater than first trimester values. These adaptations were not well explained by pregnancy-related changes in physical and cardiometabolic measures such as weight, blood pressure, lipids, and measures of inflammation and glucose metabolism. Pregnancy may represent a unique system of acute physiologic changes that maintain homeostasis during a time of acute stress. Further studies can use the data presented here as a baseline against which to assess differences in maternal vascular adaptation among women of different races, obese women, and women experiencing pregnancy complications. Understanding normal adaptation may allow for better understanding of the physiology of pregnancy complications and identify women at risk for complications early in pregnancy. If the thickened CCA IMT detected in the immediate postpartum period persists, it may help explain the higher CVD risk in women of higher parity.

3.6 TABLES AND FIGURES

Table 3.1 Unadjusted values for outcomes and key time-varying covariates by trimester

	1st Trimester n=15	2nd Trimester n=32	3rd Trimester n=37	Postpartum n=35	Overall P Value
Inter-adventitial diameter (mm)	6.47 (0.12)	6.79 (0.08)	6.89 (0.10)	6.36 (0.07)	<0.0001
CCA intima-media thickness (mm)	0.547 (0.02)	0.546 (0.01)	0.553 (0.01)	0.565 (0.01)	0.04
Weight (kg)	68.7 (2.2)	73.1 (1.6)	79.5 (1.8)	69.2 (1.5)	<0.0001
Weight change (kg)	0.55 (0.46)	7.27 (0.62)	14.4 (0.91)	4.3 (0.75)	<0.0001
Systolic blood pressure (mm Hg)	103.7 (2.1)	106.0 (1.7)	110.4 (1.4)	106.2 (1.7)	0.0005
Cardiac output (L/min)	5.24 (0.25)	5.71 (0.23)	6.23 (0.23)	4.46 (0.10)	<0.0001
Heart rate (bpm)	78.0 (2.4)	79.8 (1.6)	82.0 (1.5)	68.1 (1.5)	<0.0001
Total cholesterol (mg/dl)	201.7 (8.9)	257.3 (7.0)	273.1 (7.2)	191.4 (5.5)	<0.0001
LDL-c (mg/dl)	111.2 (7.0)	148.0 (6.3)	155.5 (6.6)	114.7 (4.8)	<0.0001
Triglycerides (mg/dl)	108.3 (10.5)	176.5 (10.2)	250.7 (13.6)	77.3 (7.0)	<0.0001
HDL-c (mg/dl)	68.8 (2.2)	74.0 (3.1)	66.8 (2.3)	61.2 (1.8)	<0.0001
Glucose (mg/dl)	79.3 (1.5)	77.2 (1.1)	77.0 (1.2)	82.6 (1.2)	0.0007
Insulin (μU/ml)	8.84 (0.78)	11.25 (0.98)	11.95 (0.81)	8.59 (0.50)	0.0001
HOMA-IR	1.64 [1.32, 2.09]	2.16 [1.56, 2.56]	2.28 [1.67, 2.51]	1.77 [1.34, 2.13]	0.01
hsCRP (mg/L)	3.58 [2.16, 5.57]	3.36 [2.31, 5.49]	3.29 [2.24, 7.01]	1.20 [.77, 2.44]	<0.0001

Normally distributed values presented as mean (SE) and P value from analysis of variance.
Skewed values presented as median [IQR] and P-value from Wilcoxon rank-sum test.

CCA is common carotid artery. Weight change is change from pre-pregnancy weight.

Table 3.2 Association between covariates and inter-adventitial diameter

	Unadjusted		Adjusted for age & pre-pregnancy BMI		Adjusted for age, pre-pregnancy BMI & weight		Adjusted for age, pre-pregnancy BMI, weight, triglycerides, Log hsCRP	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref	
Trimester 2	.361 (.07)*‡	.0001	.361 (.07)*‡	<.0001	.294 (.09)*‡	.001	.456 (.07)*‡	<.0001
Trimester 3	.498 (.07)*† ‡	.0001	.499 (.07)*†‡	<.0001	.338 (.13)*‡	.009	.683 (.12)*†‡	<.0001
Postpartum	-.015 (.05)	.74	-.014 (.05)	.76	-.032 (.05)	.49	-.029 (.04)	.44
Age (yr)			-.004 (.02)	.81	.004 (.02)	.82	-.006 (.02)	.73
Pre-pregnancy BMI (kg/m^2)			.046 (.02)	.06	.013 (.03)	.67	.006 (.03)	.85
Weight (kg)					.015 (.01)	.08	.011 (.01)	.12
Triglycerides (mg/dl)							-.002 (.00)	<.0001
Log hsCRP (mg/L)							.070 (.02)	.0002

*Different from first trimester at p<.01. †Different from second trimester at p<.05. ‡Different from postpartum at p <.01.
BMI is body mass index.

Table 3.3 Association between covariates and common carotid artery intima-media thickness

	Unadjusted		Adjusted for age, pre-pregnancy BMI		Adjusted for age, pre-pregnancy BMI, SBP & Weight Change		Adjusted for age, pre-pregnancy BMI, SBP, Weight Change & HOMA	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref	
Trimester 2	.001 (.01)	.89	.001 (.01)	.89	.018 (.01)	.17	.017 (.01)	.21
Trimester 3	.013 (.01)	.24	.013 (.01)	.24	**.041 (.02)*†**	**.046**	**.043 (.02)*†**	**.04**
Postpartum	**.027 (.01)*†**	**.02**	**.027 (.01) *†**	**.02**	**.035 (.01)***	**.005**	**.027 (.01)***	**.03**
Age (yr)			**.004 (.00)**	**.03**	.003 (.00)	.07	.002 (.00)	.15
Pre-pregnancy BMI (kg/m^2)			-.001 (.00)	.78	-.002 (.00)	.35	-.001 (.00)	.71
SBP (mm Hg)					**.001 (.00)**	**.04**	**.001 (.00)**	**.02**
Weight change (kg)					-.002 (.00)	.07	-.002 (.00)	.07
Log HOMA-IR							**-.029 (.01)**	**.02**

*Different from first trimester at p<.05. †Different from second trimester at p <.05.
BMI is body mass index. SBP is systolic blood pressure.

Table 3.4: Adventitial diameter models adjusted for age and pre-pregnancy BMI with individual physical predictors

	Unadjusted		**Adjusted for age & pre-pregnancy BMI**		**Adjusted for age, pre-pregnancy BMI, & SBP**		**Adjusted for age, pre-pregnancy BMI, & wt**		**Adjusted for age, pre-pregnancy BMI, & wt change**	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref	.84
Trimester 2	**.361 (.07)*‡**	**.0001**	**.361 (.07)*‡**	**<.0001**	**.389 (.07)*** ‡	**<.001**	**.294 (.09)*‡**	**.001**	**.321 (.09)*‡**	**<.001**
Trimester 3	**.498 (.07)*† ‡**	**<.0001**	**.499 (.07)*†‡**	**<.0001**	**.511 (.07)*** ‡	**<.001**	**.338 (.13)*‡**	**.009**	**.392 (.14)*‡**	**.008**
Postpartum	-.015 (.05)	.74	-.014 (.05)	.76	.010 (.04)	.81	-.032 (.05)	.49	-.020 (.05)	.69
Age (years)			-.004 (.02)	.81	-.003 (.02)	.83	.004 (.02)	.82	-.001 (.02)	.93
Pre-pregnancy BMI (kg/m^2)			**.046 (.02)**	**.06**	**.042 (.02)**	**.09**	.013 (.03)	.67	**.047 (.02)**	**.06**
SBP (mm Hg)					.004 (.00)	.29				
Weight (kg)							**.015 (.01)**	**.08**		
Weight change (kg)									.010 (.01)	.29

*Different from first trimester at p<.01. †Different from second trimester at p<.05. ‡Different from postpartum at p <.01.
SBP is systolic blood pressure. Weight change is from pre-pregnancy weight.

Table 3.5: Common carotid artery intima-media thickness models adjusted for age and pre-pregnancy BMI with individual physical predictors

	Unadjusted		Adjusted for age, pre-pregnancy BMI		Adjusted for age, pre-pregnancy BMI, & SBP		Adjusted for age, pre-pregnancy BMI, & weight		Adjusted for age, pre-pregnancy BMI, & weight change	
	β(SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref	
Trimester 2	.001 (.01)	.89	.001 (.01)	.89	.002 (.01)	.85	.007 (.01)	.56	.016 (.01)	.19
Trimester 3	.013 (.01)	.24	.013 (.01)	.24	.009 (.01)	.43	.022 (.02)	.22	**.042 (.02)***†	**.04**
Postpartum	**.027 (.01)*†**	**.02**	**.027 (.01) *†**	**.02**	**.026 (.01)*†‡**	**.03**	**.031 (.01)*†**	**.01**	**.036 (.01)***	**.003**
Age (years)			**.004 (.00)**	**.03**	**.004 (.00)**	**.02**	**.003 (.00)**	**.08**	**.002 (.00)**	**.11**
Pre-pregnancy BMI (kg/m^2)			-.001 (.00)	.78	-.002 (.00)	.45	.000 (.00)	.93	-.001 (.00)	.70
SBP (mm Hg)					**.001 (.00)**	**.08**				
Weight (kg)							-.000 (.00)	.66		
Weight change (kg)									**-.002 (.00)**	**.13**

*Different from first trimester at p<.05. †Different from second trimester at p <.05. ‡ Different from third trimester at p <.05.
BMI is body mass index. SBP is systolic blood pressure. Weight change is from pre-pregnancy weight.

Table 3.6: Adventitial diameter models adjusted for age, pre-pregnancy BMI, weight and individual biomarkers

	Adjusted for age, pre-pregnancy BMI & weight		**Adjusted for age, pre-pregnancy BMI, weight & total cholesterol**		**Adjusted for age, pre-pregnancy BMI, weight & HDL-c**		**Adjusted for age, pre-pregnancy BMI, weight & Triglycerides**		**Adjusted for age, pre-pregnancy BMI, weight & LDL-c**	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref	
Trimester 2	**.294 (.09)*‡**	**.001**	**.406 (.11)*‡**	**<.001**	**.307 (.09)*‡**	**<.001**	**.412 (.08)*‡**	**<.001**	**.359 (.10)**	**<.001**
Trimester 3	**.338 (.13)*‡**	**.009**	**.474 (.15)*‡**	**.002**	**.354 (.12)*‡**	**.006**	**.604 (.13)*†‡**	**<.001**	**.405 (.13)**	**.004**
Postpartum	-.032 (.05)	.49	-.044 (.05)	.34	-.030 (.05)	.55	**-.091 (.04)¥**	**.045**	-.031 (.05)	.52
Age (years)	.004 (.02)	.82	.003 (.02)	.85	.002 (.02)	.92	-.005 (.02)	.76	.003 (.02)	.86
Pre-pregnancy BMI (kg/m^2)	.013 (.03)	.67	.020 (.03)	.51	.018 (.03)	.56	.009 (.03)	.75	.019 (.03)	.54
Weight (kg)	.015 (.01)	.08	.011 (.01)	.19	.012 (.01)	.14	.011 (.01)	.13	.012 (.01)	.16
Total cholesterol (mg/dl)			-.001 (.00)	.18						
HDL-c (mg/dl)					.002 (.00)	.52				
Triglycerides (mg/dl)							**-.001 (.00)**	**.005**		
LDL-c (mg/dl)									-.001 (.00)	.38

	Adjusted for age, pre-pregnancy BMI, & weight		**Adjusted for age, pre-pregnancy BMI, weight & hsCRP**		**Adjusted for age, pre-pregnancy BMI, weight & fasting insulin**		**Adjusted for age, pre-pregnancy BMI, weight & fasting glucose**		**Adjusted for age, pre-pregnancy BMI, weight & Log HOMA-IR**	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref	
Trimester 2	**.294 (.09)*‡**	**.001**	**.315 (.08) *‡**	**<.001**	**.321(.07)*‡**	**<.001**	**.308 (.07) *‡**	**<.001**	**.319 (.07)*‡**	**<.001**
Trimester 3	**.338 (.13)*‡**	**.009**	**.332 (.12) *‡**	**.007**	**.395(.11)*‡**	**<.001**	**.380 (.11) *‡**	**<.001**	**.388 (.11)*‡**	**<.001**
Postpartum	-.032 (.05)	.49	-.022 (.04)	.61	-.043 (.05)	.34	-.034 (.05)	.45	-.051 (.05)	.27
Age (years)	.004 (.02)	.82	.004 (.02)	.84	-.003(.02)	.83	-.003 (.02)	.86	-.003(.03)	.93
Pre-pregnancy BMI (kg/m2)	.013 (.03)	.67	.011 (.03)	.72	.003 (.03)	.91	.036 (.02)	.12	.003 (.03)	.93
Weight (kg)	**.015 (.01)**	**.08**	**.014 (.00)**	**.08**	.012 (.01)	.11	**.013 (.01)**	**.07**	**.014 (.01)**	**.08**
hsCRP (mg/L)			**.004 (.00)**	**.03**						
Fasting insulin (µU/ml)					.004 (.01)	.55				
Fasting glucose (mg/dl)							-.005 (.00)	.13		
Log HOMA-IR									-.013 (.07)	.86

*Different from first trimester at p<.01. †Different from second trimester at p<.05. ‡Different from postpartum at p <.01. ¥Different from first trimester at p <.05.

Table 3.7: Common carotid artery intima media thickness models adjusted for age, pre-pregnancy BMI, systolic blood pressure, weight change and individual biomarkers

	Adjusted for age, pre-pregnancy BMI, SBP and weight change		**Adjusted for age, pre-pregnancy BMI, SBP, weight change & total cholesterol**		**Adjusted for age, pre-pregnancy BMI, SBP, weight change & HDL-c**		**Adjusted for age, pre-pregnancy BMI, & Triglycerides**		**Adjusted for age, pre-pregnancy BMI, & LDL-c**	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref	
Trimester 2	.018 (.01)	.17	.016 (.02)	.31	.016 (.01)	.24	.013 (.01)	.37	.017 (.01)	.26
Trimester 3	**.041 (.02)*†**	**.046**	**.040 (.02)**	**.09**	**.038 (.02)**	**.07**	.035 (.02)	.15	**.041 (.02)** †	**.06**
Postpartum	**.035 (.01)***	**.005**	**.030 (.01)*** †	**.02**	**.028 (.01)**	**.04***	**.031 (.01)***	**.02**	**.031 (.01)***	**.02**
Age (years)	**.003 (.00)**	**.07**	**.003 (.00)**	**.09**	**.003 (.00)**	**.08**	**.003 (.00)**	**.09**	**.003 (.00)**	**.08**
Pre-pregnancy BMI (kg/m^2)	-.002 (.00)	.35	-.002 (.00)	.38	-.002 (.00)	.34	-.003 (.00)	.24	-.002 (.00)	.39
SBP (mmHg)	**.001 (.00)**	**.04**	**.001 (.00)**	**.05**	**.001 (.00)**	**.04**	**.001 (.00)**	**.01**	**.001 (.00)**	**.05**
Weight change (kg)	**-.002 (.00)**	**.07**	**-.002 (.00)**	**.05**	**-.002 (.00)**	**.04**	**-.003 (.00)**	**.03**	**-.003 (.00)**	**.04**
Total cholesterol (mg/dl)			-.000 (.00)	.95						
HDL-c (mg/dl)					-.000 (.00)	.39				
Triglycerides (mg/dl)							.000 (.00)	.45		
LDL-c (mg/dl)									-.000 (.00)	.80

*Different from first trimester at p<.05. †Different from second trimester at p <.05.
BMI is body mass index. SBP is systolic blood pressure. Weight change is from pre-pregnancy.

Table 3.7 Continued: Common carotid artery intima media thickness models adjusted for age, pre-pregnancy BMI, systolic blood pressure, weight change and individual biomarkers

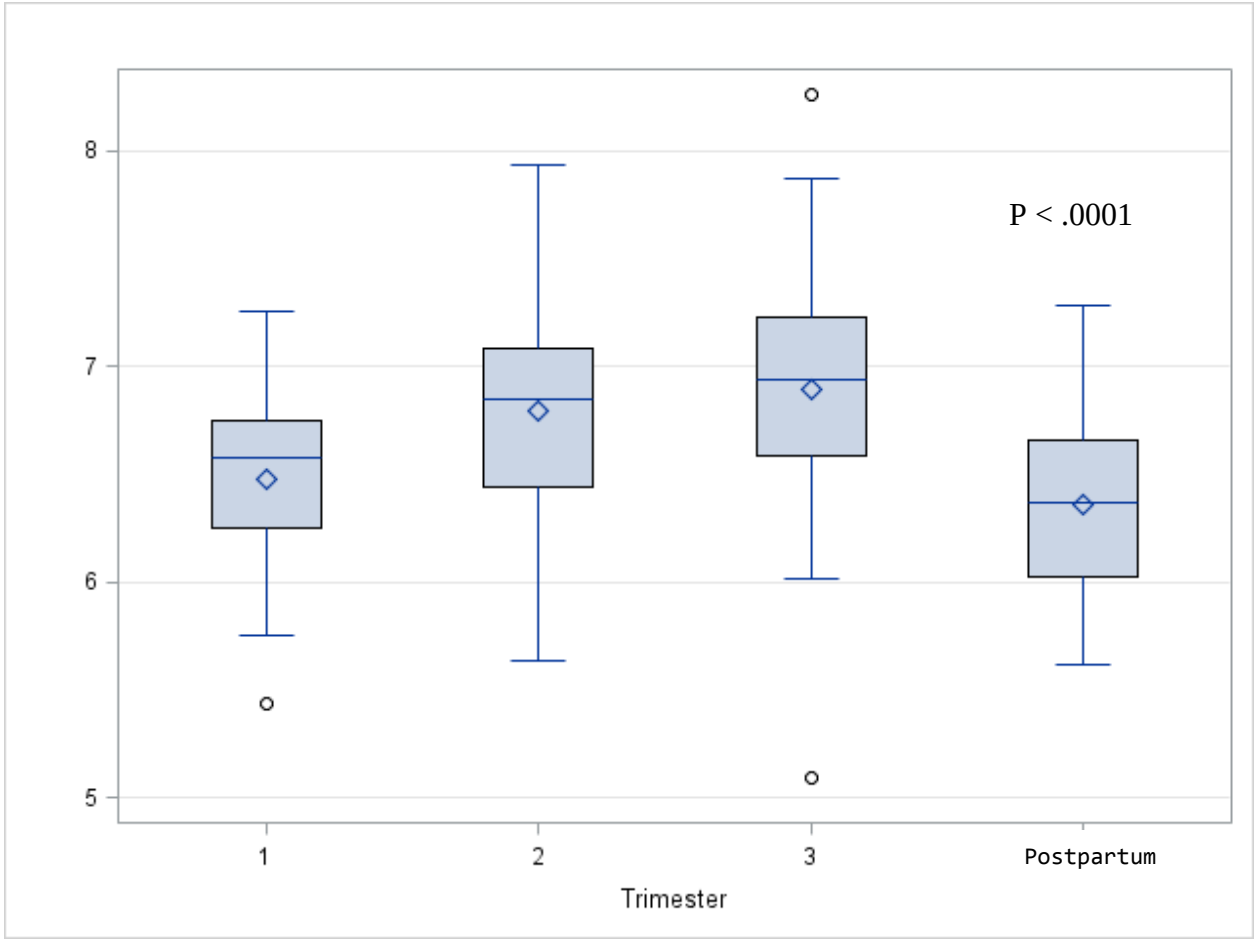
	Adjusted for age, pre-pregnancy BMI, SBP and weight change		**Adjusted for age, pre-pregnancy BMI, SBP, weight change & hsCRP**		**Adjusted for age, pre-pregnancy BMI, SBP, weight change & fasting insulin**		**Adjusted for age, pre-pregnancy BMI, & fasting glucose**		**Adjusted for age, pre-pregnancy BMI, & log HOMA-IR**	
	B (SE)	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**	**B (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref	
Trimester 2	.018 (.01)	.17	.016 (.01)	.24	.017 (.01)	.22	.013 (.01)	.33	.017 (.01)	.21
Trimester 3	**.041 (.02)*†**	**.046**	**.040 (.02) †**	**.06**	**.042 (.02) *†**	**.04**	**.039 (.02) †**	**.06**	**.043 (.02)*†**	**.04**
Postpartum	**.035 (.01)***	**.005**	**.032 (.01)***	**.01**	**.027 (.01)***	**.03**	**.032 (.01)***	**.01**	**.027 (.01)***	**.03**
Age (years)	**.003 (.00)**	**.07**	**.003 (.00)**	**.08**	.003 (.00)	.13	**.003 (.00)**	**.06**	.002 (.00)	.15
Pre-pregnancy BMI (kg/m^2)	-.002 (.00)	.35	-.002 (.00)	.35	-.003 (.00)	.42	-.002 (.00)	.43	-.001 (.00)	.71
SBP (mmHg)	**.001 (.00)**	**.04**	**.001 (.00)**	**.04**	**.001 (.00)**	**.01**	**.001 (.00)**	**.04**	**.001 (.00)**	**.02**
Weight change (kg)	**-.002 (.00)**	**.07**	**-.003 (.00)**	**.04**	**-.003 (.00)**	**.06**	**-.002 (.00)**	**.04**	**-.002 (.00)**	**.07**
hsCRP (mg/L)			.000 (.00)	.54					**-.029 (.01)**	**.02**
Fasting insulin (µU/ml)					-.002 (.00)	.13				
Fasting glucose (mg/dl)							**-.001 (.00)**	**.09**		
Log HOMA-IR									**-.029 (.01)**	**.02**

*Different from first trimester at p<.05. †Different from second trimester at p <.05.
BMI is body mass index. SBP is systolic blood pressure. Weight change is from pre-pregnancy.

Table 3.8 Number of weeks postpartum as a continuous predictor

	Unadjusted		Adjusted for age and pre-pregnancy BMI		Fully Adjusted*	
	ß (SE)	**p-value**	**ß (SE)**	**p-value**	**ß (SE)**	**p-value**
CCA IMT	-.005 (.00)	.28	-.003 (.00)	.42	-.007 (.00)	.11
IAD	.009 (.04)	.81	.018 (.02)	.63	-.006 (.04)	.87

*Fully adjusted model for IMT: age, pre-pregnancy BMI, SBP, weight change, log HOMA-IR.
Fully adjusted model for IAD: age, pre-pregnancy BMI, SBP, triglycerides, log CRP.
CCA IMT is common carotid artery intima media thickness. IAD is inter-adventitial diameter. BMI is body mass index (kg/m^2).



All pairwise comparisons significant at p < .0001 EXCEPT: Trimesters 1 vs. postpartum p = .76, Trimesters 2 vs. 3 p = .03. Adjusted for age and pre-pregnancy body mass index.

Figure 3.1 Changes in inter-adventitial diameter throughout pregnancy cycle

CCA IMT (mm)

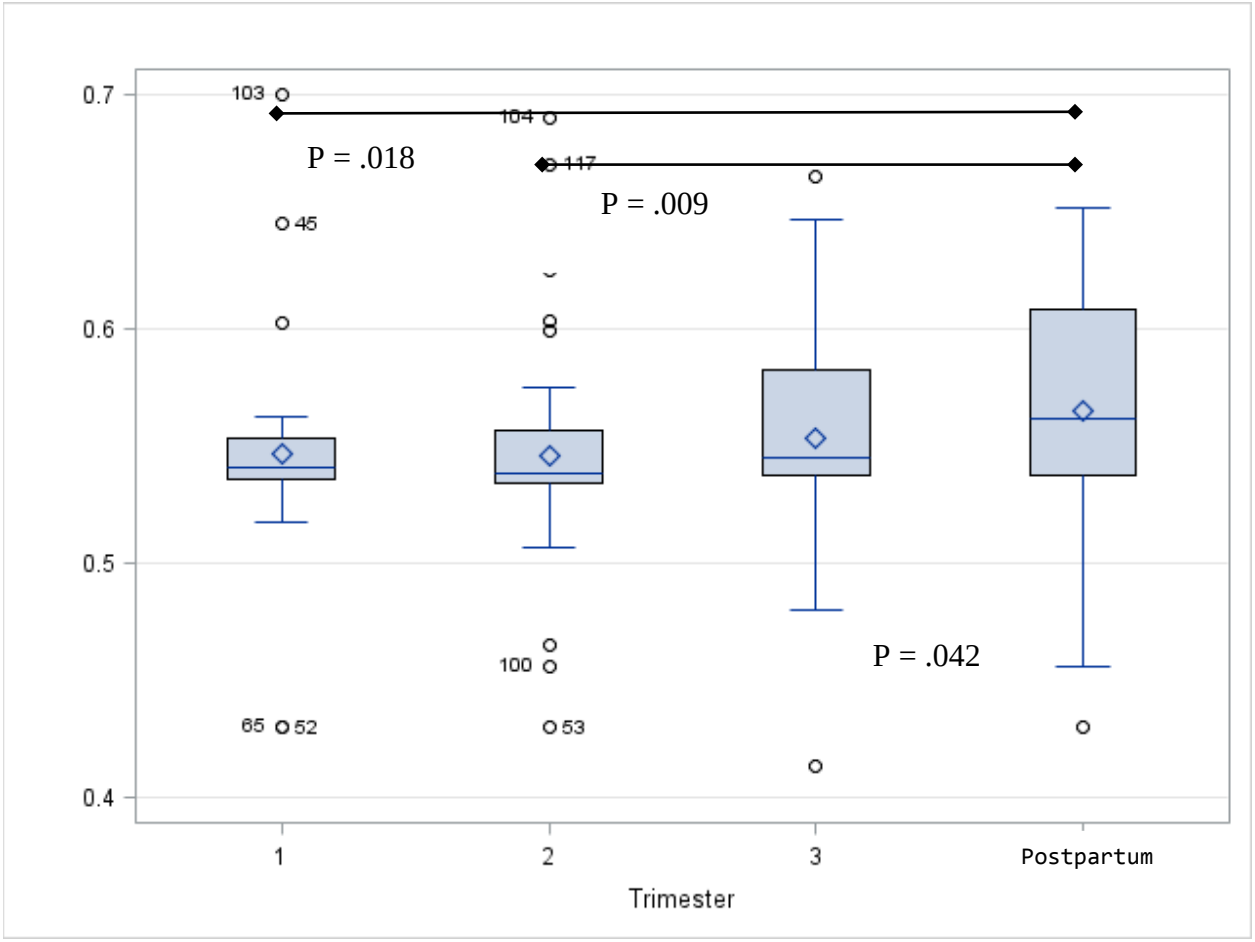


Figure 3.2. Changes in CCA IMT throughout pregnancy cycle, adjusted for maternal age and pre-pregnancy BMI

4.0 BRACHIAL ARTERY STIFFENS DURING THE PREGNANCY CYCLE IN HEALTHY PRIMIGRAVIDAS

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4.1 ABSTRACT

Objectives: Higher parity is associated with greater subclinical cardiovascular disease (CVD) in mid-life and older women, and with higher risk of CVD overall. Prospective studies of arterial change throughout normal pregnancy are lacking; without them it is unclear whether unhealthy changes of the vasculature during pregnancy persist postpartum and raise women's risk of CVD. The goal of this study was to prospectively assess normal vascular adaptation in healthy pregnant women. Our hypotheses were that during the course of healthy pregnancy brachial artery distensibility will not change.

Methods: The Maternal Vascular Adaptations to Healthy Pregnancy Study (Pittsburgh, PA, 2010-2013) assessed 43 healthy women during each trimester of their first pregnancy and 6-8 weeks postpartum. Brachial artery distensibility was measured using the DynaPulse wave form analyzer. Linear mixed models were used to compare brachial artery distensibility at each time point, after adjustment for age and pre-pregnancy body mass index. Various physical and cardiometabolic measures were then considered as mediators of the relationship between pregnancy and brachial artery distensibility.

Results: There were 37 women (age 28.2 ± 4.5 years, pre-pregnant BMI 24.4 ± 3.2 kg/m^2) with uncomplicated pregnancies. Mean (SE) brachial artery distensibility (%/mmHg) decreased from 7.64 (0.28) 1st trimester to 6.84 (0.21) 3rd trimester ($p < .01$) and then remained unchanged at 6.82 (0.21) postpartum. In multivariable models weight gain and increased cardiac output were significantly related to increased stiffness.

Conclusions: Contrary to our hypotheses, the brachial artery stiffened during pregnancy and remained stiffer 6-8 weeks postpartum. Increased weight and cardiac output of pregnancy are associated with this change. Whether this indicates that persistence of specific vascular effects of pregnancy may inform long term CVD risk remains to be explored.

4.2 INTRODUCTION

Higher parity (number of births) is related to higher cardiovascular disease (CVD) risk in women,[75-77] but the mechanisms for this higher risk are unknown. It has long been thought that pregnancy's acute effects on the vasculature are positive; both systemic vascular resistance and global (combined central and peripheral) arterial stiffness decrease during pregnancy.[88,181] Recent studies, however, suggest that the effects of pregnancy on the vasculature are more mixed. They have found that the carotid artery actually stiffens during the course of pregnancy,[171,194] is stiffer in pregnant women at greater gestational ages,[206] and is stiffer in mid-life and older women of higher parity.[207] A recent study in elderly people showed that carotid stiffness is associated with greater risk of CVD;[45] these findings may help explain the link between higher parity and greater CVD risk. However, it is important to note that these studies were limited by lack of prospective data collection throughout pregnancy,[206,207] small sample size,[171,194] and lack of collection of data on hemodynamic and metabolic changes during pregnancy.[171,194,206,207]

Stiffness of the brachial artery has been shown to be related to CVD risk factors in young adults,[208] but the effects of pregnancy on stiffness of the brachial artery have not been described. Distensibility of the brachial artery, a measure of peripheral arterial stiffness, can be measured easily, reliably and non-invasively using the DynaPulse system (Pulse Metric, Inc., San Diego, CA).[20] The DynaPulse has the advantage of simultaneously measuring several hemodynamic parameters known to change during pregnancy, including cardiac output, and thus can be used to gather prospective information on both changes in arterial stiffness during pregnancy and factors associated with those changes. Understanding factors associated with changes in stiffness of the brachial artery during pregnancy may illuminate the relationship between pregnancy and CVD risk.

An objective of the Maternal Vascular Adaption to Healthy Pregnancy (MVP) Study was to evaluate prospectively the normal changes in brachial artery distensibility that occur during uncomplicated first pregnancy. A secondary objective was to explore the relationship between pre-conception and pregnancy-related factors and brachial artery distensibility. We hypothesized that despite increases in fluid volume and weight, factors associated with higher arterial stiffness,[209] the brachial artery would not stiffen during pregnancy.

4.3 METHODS

4.3.1 MVP Study Design and Population

The MVP study prospectively assessed brachial artery distensibility in a cohort of healthy women experiencing their first pregnancies. Participants were healthy non-smoking women, age

21-39 years, at less than 38 weeks gestational age in their first pregnancies. Exclusion criteria were: 1) vasoactive medication use; 2) infertility history, defined as ever having had a period of at least 12 months of inability to achieve pregnancy, or using fertility medications to achieve pregnancy; 3) family history of premature coronary artery disease; and 4) multiple gestation. Participants were recruited with mailings and flyers distributed to the University of Pittsburgh community, use of University research registries, and advertisements in the offices of local (Pittsburgh, PA) prenatal care providers and on the internet. All participants signed an informed consent document approved by the University of Pittsburgh Institutional Review Board.

Study visits were scheduled at 12-14 weeks, 24-26 and 36-38 weeks of pregnancy, and at 6-8 weeks postpartum, and occurred between June 2010 and March 2013. After a telephone screening for eligibility, women could begin the study at any one of the pregnancy visits. At each visit research staff obtained physical measures and staff of the University of Pittsburgh Ultrasound Research Laboratory obtained DynaPulse measurements of cardiovascular parameters. Staff reviewed prenatal and birth records after the final study visit. The targeted sample size of 46 women assumed that 10-20% of women would develop a pregnancy complication and that there would be 25% attrition from the study. The study enrolled 44 women, of whom 43 had multiple visits and 6 developed pregnancy complications, leaving 37 women in the sample for this analysis. This provided greater than 80% power to detect a 0.5 SD difference, given an assumed 0.5 correlation among the repeated observations.

4.3.2 Brachial Artery Distensibility and Cardiac Output

Brachial artery distensibility and cardiac output were measured using the DynaPulse 5000A (Pulse Metric, Inc, San Diego, CA) by vascular sonographers from the University of

Pittsburgh Ultrasound Research Laboratory. Participants rested in a seated position for 5 minutes and then a standard cuff sphygmomanometer was attached to the left arm. The cuff was automatically inflated for a calibration run; after calibration 3 runs were performed, and the mean of the 3 used for the analysis. The offline waveform analysis of arterial pressure signals performed by PulseMetric, Inc. uses validated models of the vascular system to calculate cardiovascular parameters.[20] The brachial artery distensibility coefficient is calculated as change in volume divided by change in pressure, or as $4\pi/(dP/dt_{pp}*t_{pp})$, where dP/dt_{pp} is the amplitude from the peak positive pressure derivative to the peak negative pressure derivative of oscillometric pulsation signal and t_{pp} is the interval between the peak positive and peak negative pressure derivatives.[208] To calculate cardiac output, dP/dt_{pp} was used to approximate left ventricular $dP/dt_{pp,}$ and a Gaussian transformation function was then used to calculate left ventricular contractility (LVC). Cardiac output was calculated as cardiac output = LVC * heart rate * body surface area. Body surface area was calculated as a function of weight and height.[210] Validation studies of the methods have been published, with a linear regression correlation of r = 0.83 for compliance compared with measurements from intraarterial cannulation.[20]

4.3.3 Demographic, Physical and Laboratory Measures

Women completed a self-administered demographic form with information on age, race/ethnicity, marital status, education, employment, income, insurance status and intended birth site at their initial study visit. Each visit included physical measures, vascular evaluation, and laboratory assays. Research staff measured height with the participant not wearing shoes using a stadiometer, and the mean of 2 readings from the first visit used. Lightly clothed participants were weighed on a standard balance scale and the mean of 2 readings used. Pre-

pregnancy weight was identified as the pre-pregnancy weight documented in the prenatal record or, if not available, as a documented weight in the health record in the three months prior to the last menstrual period. Pre-pregnancy body mass index (BMI) was calculated as pre-pregnancy weight in kilograms divided by height in meters squared. Weight change was calculated as the difference between weight at each study visit and pre-pregnancy weight.

Blood pressure was measured according to standardized protocol. After participants rested for 5 minutes in a quiet room, research staff palpated the right radial pulse for 30 seconds and then measured blood pressure in that arm using a mercury sphygmomanometer, with auscultation of the 5th Korotkoff sound indicating diastolic pressure. The mean of the last 2 of 3 measurements was used for the analysis. Data on demographic and physical measures and from records reviews were collected and managed using REDCap electronic data capture tools hosted at the University of Pittsburgh.[195] REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Laboratory assays were performed on fasting serum samples at the Heinz Nutrition Laboratory at the University of Pittsburgh Graduate School of Public Health (Pittsburgh, PA). Total cholesterol,[196] high density lipoprotein (HDL-c),[197] low density lipoprotein (LDL-c)[198], triglycerides[199] and glucose[200] were determined using standard laboratory procedures. Insulin was measured using standard radio-immune assay (Linco Research, St. Charles, MO). HOMA-IR, a measure of insulin resistance, was calculated as (glucose x insulin)/405. [201] High-

sensitivity C-reactive protein (hsCRP) was measured with an enzyme-linked immunoassay (Alpha Diagnostics International, Inc. San Antonio, TX).

4.3.4 Statistical Analysis

Distributions were assessed for all variables. Measures with approximately normal distributions are presented as means ± standard deviations (SD); measures not normally-distributed are presented as medians with interquartile range, and categorical variables are presented as percentages. hsCRP and HOMA-IR were skewed and were log-transformed to meet the linear assumptions of regression modeling. Longitudinal measures that were collected over time (brachial artery distensibility, physical, and laboratory measures) are presented for each of the 4 time points in the study. Linear mixed models were used to calculate 1) estimated mean brachial artery distensibility adjusted for maternal age and pre-pregnancy BMI, and indicators of each trimester and postpartum; 2) the overall effect of time in the pregnancy cycle on distensibility; and 3) which specific time points (trimester, postpartum) vary from each other.

To determine associations between cardiovascular risk factors and distensibility a hierarchical regression analysis was used. Models were constructed including time-varying systolic blood pressure, weight, weight change, heart rate and cardiac output. Maternal age and pre-pregnancy BMI were a priori included in all models. Significant predictors (systolic blood pressure, weight, weight change and cardiac output) were included in the full models and, and statistically significant predictors were retained in a best model. Next, biomarkers were tested individually in the best model. Biomarkers with statistical significance (glucose, HDL, triglycerides and hsCRP) were then modeled together, and only statistically significant factors were retained. A sensitivity analysis was performed eliminating 3 outliers for hsCRP, and

another was done including only the 15 women who completed all study visits. To assess whether distensibility changes over the course of the postpartum period, simple linear regression was performed using weeks postpartum as a continuous predictor of brachial artery distensibility.

A two-sided p-value of <.05 was considered statistically significant. All analyses were performed using SAS statistical software Windows 9.3 (SAS Institute, Cary, NC).

4.4 RESULTS

Participants' average age at baseline was 28.4 ± 4.6 years and average BMI was 24.3 ± 3.3 (kg/m^2). A majority were white (91.9%), married or living as married (89.2%), well-educated (89.2% college graduate or greater) and employed (64.9% full time, 24.3% part time). The mean number of visits was 3.3 (range 2-4), with 15 women completing all 4 visits.

The brachial artery stiffened between the first and second trimesters, and remained stiffer into the postpartum period. Distensibility decreased from 7.50 %/mm Hg in the 1st trimester to 7.01 %/mm HG 2nd in the second trimester, and then remained stable into the postpartum period (overall P = .049) (Table 4.1). After adjustment for maternal age and pre-pregnancy BMI, this pattern remained the same (P=.035) (Figure 4.1).

Changes in weight, blood pressure, heart rate, cardiac output, and lipid, glucose and hsCRP concentrations followed expected patterns for healthy pregnancies (Table 4.1). When weight and hemodynamic factors were assessed, lower brachial artery distensibility was associated with higher systolic blood pressure, higher weight, greater weight change, and greater cardiac output (Table 4.3). When modeled together, greater weight gain and cardiac output remained significantly associated with a stiffer brachial artery (Table 4.2). These substantially

changed the pattern of change in arterial stiffness seen throughout pregnancy (Table 4.2), with only the postpartum value becoming significantly different from the 1st trimester value.

When metabolic factors were considered individually (Table 4.4) higher HDL-c concentrations were associated with a stiffer brachial artery and higher triglycerides, glucose, and hsCRP concentrations were associated with lower stiffness. After modeling the significant metabolic factors together, only hsCRP remained significantly associated with lower stiffness (Table 4.2). Including hsCRP substantially changed the beta coefficient for the postpartum period, from -0.735 (0.01) to -0.368 (.29). Results of all analyses were consistent of those from the sensitivity analysis including only the women who completed all 15 study visits (data not shown).

Final visits were conducted at 6-13 weeks postpartum, with a mean of 8.6 weeks. At the postpartum visit, 88% of women were fully breastfeeding their infants. Number of weeks postpartum was not significantly related to distensibility (data not shown).

4.5 DISCUSSION

This study evaluated changes in brachial artery distensibility during the course of normal first pregnancy, and found that the brachial artery stiffened between the first and second trimesters, and then remained stiffer than first trimester values at 6 to 8 weeks postpartum. This was contrary to our hypothesis that the artery would not stiffen during pregnancy. These changes were associated with greater weight gain and increase in cardiac output during pregnancy, suggesting these factors mediate the change. Brachial artery distensibility was not

associated with blood pressure independent of changes in weight, and was associated with several markers of inflammation, including hsCRP, triglyceride, and glucose concentrations.

These findings support the observation that the pregnancy's effect on the vasculature differs by vascular bed. While overall systemic resistance decreases and the aorta becomes more compliant during pregnancy, mounting evidence shows that the carotid artery stiffens.[171,194,206,207] Our study found that the brachial artery also stiffened during healthy pregnancy. The mechanism for the different effects on different arterial beds is unknown. It has been speculated that the carotid artery and aorta may express different proportions of receptors for estrogen and angiotensin II,[171] and that this may explain the varied effects of pregnancy.

These findings are consistent with research showing that change in arterial stiffness is strongly related to weight change in young adults. In an observational study, weight change over a 2 year period was significantly related to change in aortic stiffness in 20-40 year old adults, independent of aging and changes in blood pressure.[209] A clinical trial examining a weight loss intervention coupled with a low sodium diet found that weight loss over a 6 month period was associated with decreased arterial stiffness, again independent of changes in blood pressure.[188] The mechanism of these changes is unknown, but researchers have speculates that weight gain is independently related to increased vascular stiffness.[211] Our study measured cardiac output, and strongly suggests that in pregnancy weight gain and increased cardiac output were related to the increased arterial stiffness. Consistent with findings from the Cardiovascular Risk in Young Finns study,[206] lipid concentrations were not independently related to arterial stiffness.

There are several theories explaining the relationship between high parity and higher risk of CVD, including persistence of acute changes of pregnancy,[85,174] long-term effects of accumulated CVD risk factors following pregnancies,[67,69,70] and confounding based on

socioeconomic and lifestyle factors.[81,212] Our findings suggest that persistence of an acute pregnancy change, stiffening of the brachial artery, may be one piece of the equation. The studies cited above[209] suggest that weight loss after pregnancy should result in improved arterial stiffness, but it is unknown if there will be a complete reversal. Some mechanisms of stiffening, such as changes in endothelial function, are reversible; others, however, including fragmentation of elastin or changes in collagen and protein cross-linking may not be.[21,209] Persistent greater arterial stiffness may contribute to higher CVD risk largely through its effects on increasing systolic blood pressure.[21] Long term follow up studies are needed to determine whether the arterial stiffening seen during pregnancy persists.

The finding that brachial artery stiffness was negatively related to several measures of inflammation, while HDL-c was related to higher stiffness, was unexpected. Perhaps the hormonal milieu of pregnancy, which includes very high levels of sex hormones and relaxin, may have a protective effect on the vasculature from what may normally be negative metabolic changes.

These findings also may have implications for research into pregnancy complications, particularly preeclampsia. Preeclampsia is a pregnancy-specific disease characterized by new onset hypertension after 20 weeks of pregnancy and proteinuria. Peripheral arterial stiffness in pregnancy has been found to be higher in pregnancies complicated by preeclampsia than in healthy pregnancies in some,[179,180] but not all studies,[161] and at 16 months postpartum.[183] Our findings provide baseline data that can be used in future research examining patterns of arterial stiffness in healthy pregnancies compared to pregnancies complicated by preeclampsia. The DynaPulse device is easily used in the outpatient setting, and could be a useful clinical tool.

This study was to our knowledge the first to prospectively follow a cohort of pregnant women into the postpartum period with a reliable measure of brachial artery distensibility and concurrent collection of various hemodynamic and metabolic measures. It benefited from high retention of participants. However, there were some notable limitations to the study. It was limited by the timing of the visits. The first visit did not occur until 12-14 weeks of pregnancy. Substantial hemodynamic changes occur as early as 5 weeks of pregnancy,[88] so it is likely that our first visit does not represent a true pre-pregnancy baseline. Pre-pregnancy weights were largely self-reported; it has been shown that women tend to under-report pre-pregnant weight.[213] The postpartum visit was performed at an average of 8 weeks postpartum. It is likely that not all of the hemodynamic changes of pregnancy have resolved at that point[90] and longer follow-up is required to determine if the increased brachial artery stiffness persists. Participants were overwhelmingly breastfeeding at that point, and the results might not reflect those in women formula feeding their infants. The power calculations for the study were based on the assumption that women would attend all 4 study visits; in actuality, women attended an average of 3.3 visits, decreasing the power of the study. However, the change in brachial artery distensibility was greater than that posited, providing sufficient power to detect significant differences. The sensitivity analysis including only the 15 women completing all visits replicated all findings, confirming that failure to attend all the study visits did not negatively affect the results.

The largely white, well-educated women in this cohort may not be representative of the general population of first time pregnant women, yet the study has utility as providing baseline data against which arterial stiffness changes in other demographic groups can be assessed.

The results of this study apply only to the brachial artery, and cannot be assumed to apply to the rest of the arterial bed, which has indeed been demonstrated to have increased compliance during pregnancy. Brachial artery distensibility is a less studied measure than the more frequently used pulse wave velocity, and its relationship to future CVD is not well understood, although it is associated with coronary artery calcification, and independent predictor of CVD risk.[214] Brachial artery distensibility has rarely been studied in pregnancy, and validity and reliability of the measure in pregnancy are not well understood. Yet, if these are demonstrated, the measure may have great utility because of its ease of use.

We found that the brachial artery stiffens between the first and second trimesters of a healthy first pregnancy, and remains stiffer at 6-8 weeks postpartum. This change appears to be explained by the increased weight and cardiac output that occur during pregnancy, and is independent of changes in blood pressure. Several inflammatory markers are associated with greater stiffening, but after multivariable adjustment only hsCRP was independently associated. This stiffening is similar to stiffening demonstrated in the carotid artery, but different from the increased compliance seen in the aorta. Understanding normal arterial changes may allow for better understanding of the physiology of pregnancy complications and identify women at risk for complications early in pregnancy. The relationship between parity and future CVD is complex and likely multifactorial, however if this stiffening of the brachial artery persists, it may help explain the greater CVD risk in women of higher parity.

4.6 TABLES AND FIGURES

Table 4.1 Unadjusted values for outcomes and key time-varying covariates by trimester

	1st Trimester n=17	2nd Trimester n=32	3rd Trimester n=37	Postpartum n=35	P value
BrachD (%/mm Hg)	7.50 (.20)	7.01 (.21)	6.93 (.22)	6.95 (.17)	.049
Weight (kg)	68.7 (2.2)	73.1 (1.6)	79.5 (1.8)	69.2 (1.5)	<.0001
Weight change(kg)	.55 (.46)	7.27 (.62)	14.4 (.91)	4.3 (.75)	<.0001
Systolic blood pressure (mm Hg)	103.7 (2.1)	106.0 (1.7)	110.4 (1.4)	106.2 (1.7)	.0005
Diastolic blood pressure (mm Hg)	61.8 (1.6)	61.3 (1.2)	68.4 (1.2)	68.7 (1.2)	<.0001
Pulse pressure (mm Hg)	42.2 (1.9)	44.3 (1.5)	41.9 (1.5)	37.6 (1.5)	<.0001
Cardiac Output (L/min)	5.24 (.25)	5.71 (.23)	6.23 (.23)	4.46 (.10)	<.0001
Heart Rate (bpm)	78.0 (2.4)	79.8 (1.6)	82.0 (1.5)	68.1 (1.5)	<.0001
Total Cholesterol (mg/dl)	201.7 (8.9)	257.3 (7.0)	273.1 (7.2)	191.4 (5.5)	<.0001
LDL-c (mg/dl)	111.2 (7.0)	148.0 (6.3)	155.5 (6.6)	114.7 (4.8)	<.0001
Triglycerides (mg/dl)	108.3 (10.5)	176.5 (10.2)	250.7 (13.6)	77.3 (7.0)	<.0001
HDL-c (mg/dl)	68.8 (2.2)	74.0 (3.1)	66.8 (2.3)	61.2 (1.8)	<.0001
Glucose (mg/dl)	79.3 (1.5)	77.2 (1.1)	77.0 (1.2)	82.6 (1.2)	.0007
Insulin (µU/ml)	8.84 (.78)	11.25 (.98)	11.95 (.81)	8.59 (.50)	.0001
HOMA-IR*	1.64 [1.32, 2.09]	2.16 [1.56, 2.56]	2.28 [1.67, 2.51]	1.77 [1.34, 2.13]	.01
HS-CRP* (mg/L)	3.58 [2.16, 5.57]	3.36 [2.31, 5.49]	3.29 [2.24, 7.01]	1.20 [.77, 2.44]	<.0001

Values presented as mean (SE) or median [IQR].
*p-values from Wilcoxon rank-sum test
BrachD is brachial artery distensibility. Weight change is from pre-pregnancy.

Table 4.2 Association between covariates and brachial artery distensibility coefficient

Main Effect and Covariates	Unadjusted		Minimally Adjusted		Final Model	
	β (SE)	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**
Trimester 1	Ref		Ref		Ref	
Trimester 2	**-0.581 (.27)***	**0.03**	**-0.595 (.27)***	**0.03**	0.214 (.30) ‡	0.48
Trimester 3	**-0.711 (.26)***	**0.01**	**-0.732 (.26)***	**0.01**	0.846 (.42) †‡	0.05
Postpartum	**-0.720 (.28)***	**0.01**	**-0.744 (.27)***	**0.01**	**-0.735 (.26)***	**0.01**
Baseline Age (yr)			0.000 (.03)	0.997	**-0.076 (.03)**	**0.02**
Pre-pregnancy BMI (kg/m^2)			-0.086 (.05)	0.08	0.002 (.05)	0.96
Weight change (kg)					**-0.074 (.03)**	**0.01**
Cardiac Output (L/min)					**-0.486 (.10)**	**<0.0001**

*Different from first trimester at p<.05. †Different from second trimester at p<.05. ‡Different from postpartum at p <.01.
** Without CRP outliers.
Model 1: Adjusted for age & pre-pregnancy BMI
Model 2: Adjusted for age, pre-pregnancy BMI, weight change & cardiac output
Model 3: Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & Log HS-CRP**
BMI is body mass index. Weight change is from pre-pregnancy.

Table 4.3: Brachial artery distensibility coefficient models adjusted for age and pre-pregnancy BMI with individual predictors

	Adjusted for age & pre-pregnancy BMI		**Adjusted for age, pre-pregnancy BMI, & SBP**		**Adjusted for age, pre-pregnancy BMI, & wt**		**Adjusted for age, pre-pregnancy BMI & wt change**		**Adjusted for age, pre-pregnancy BMI & cardiac output**		**Adjusted for age, pre-pregnancy BMI & heart rate**	
	β (SE)	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref¥		Ref	
Trimester 2	**-.595 (.27)**	**.03**	**-.587 (.26)***	**.03**	-.315 (.29)	.28	-.047 (.32)	.88	-.211 (.25) ¥	.40	**-.638 (.27)***	**.02**
Trimester 3	**-.732 (.26)***	**.01**	-.533 (.28)	.06	-.032 (.38)‡	.93	.491 (.47) ¥	.30	-.080 (.25) ¥	.75	**-.727 (.27)***	**.01**
Postpartum	**-.744 (.27)***	**.01**	**-.707 (.28)***	**.01**	**-.626 (.28)***	**.03**	-.462 (.29)	.12	**-1.00 (.24)**	**<.0001**	**-.809 (.30)***	**.01**
Age (yr)	.000 (.03)	.997	-.011 (.03)	.75	-.031 (.04)	.38	-.029 (.03)	.38	-.057 (.03)	.08	.001 (.03)	.99
Pre-pregnancy BMI (kg/m^2)	-.086 (.05)	.08	-.040 (.05)	.44	.046 (.07)	.51	-.087 (.05)	.06	.015 (.05)	.75	.128 (.05)	.13
SBP (mm Hg)			**-.030 (.01)**	**.02**								
Weight (kg)					**-.054 (.02)**	**.01**						
Weight change (kg)							**-.091 (.03)**	**.00**				
Cardiac output (L/min)									**-.539 (.10)**	**<.0001**		
Heart rate (bpm)											-.002 (.01)	.87

*Different from first trimester at p<.05. †Different from second trimester at p<.05. ‡Different from postpartum at p <.05. ¥Different from postpartum at p <.01.
BMI is body mass index. SBP is systolic blood pressure. Weight change is from pre-pregnant.

Table 4.4: Brachial artery distensibility coefficient models adjusted for age, pre-pregnancy BMI, weight change and cardiac output with individual biomarkers

	Adjusted for age & pre-pregnancy BMI		**Adjusted for age, pre-pregnancy BMI, weight change and cardiac output**		**Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & total cholesterol**		**Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & total HDL**		**Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & triglycerides**		**Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & LDL**	
	β (SE)	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref		Ref	
Trimester 2	**-.595 (.27)***	**.03**	.214 (.30) ‡	.48	.360 (.35)	.31	.423 (.31)	.17	.142 (.33)	.66	.378 (.33)	.26
Trimester 3	**-.732 (.26)***	**.01**	.846 (.42) †‡	.05	**.985 (.47)*** †¥	**04**	**.922 (.42)*¥**	**.03**	.621 (.51) ‡	.22	**1.00 (.45)*** †¥	**.03**
Postpartum	**-.744 (.27)***	**.01**	**-.735 (.26)***	**.01**	**-.579 (.28)*** †	**.04**	**-.073 (.27)*¥**	**.01**	-.042 (.28)	.15	**-.575 (.28)***†	**.04**
Age (yr)	.000 (.03)	.997	**-.076 (.03)**	**.02**	**-.077 (.03)**	**.01**	**-.074 (.03)**	**.03**	-.074 (.03)	.66	**-.077 (.03)**	**.02**
Pre-preg BMI (kg/m^2)	-.086 (.05)	.08	.002 (.05)	.96	-.006 (.05)	.89	-.012 (.05)	.81	-.015 (.05)	.76	-.006 (.05)	.90
Weight change (kg)			**-.074 (.03)**	**.01**	**-.076 (.03)**	**.00**	**-.076 (.03)**	**.00**	**-.087 (.03)**	**.00**	**-.076 (.03)**	**.00**
Cardiac output (L/min)			**-.486 (.10)**	**<.0001**	**-.472 (.10)**	**<.0001**	**-.473 (.10)**	**<.0001**	**-.386 (.10)**	**.0004**	**-.471 (.10)**	**<.0001**
Total cholesterol (mg/dl)					-.000 (.00)	.97						
HDL-c (mg/dl)							**-.017 (.01)**	**.02**				
Triglycerides (mg/dl)									**.003 (.00)**	**.045**		
LDL-c (mg/dl)											-.001 (.00)	.83

*Different from first trimester at p<.05. †Different from second trimester at p<.05. ‡Different from postpartum at p <.05. ¥Different from postpartum at p <.01.
Pre-preg BMI is pre-pregnancy body mass index, weight change is from pre-pregnancy, HDL-c is high density lipoprotein concentration, LDL-c is low density lipoprotein concentration.

Table 4.4 Continued: Brachial artery distensibility coefficient models adjusted for age, pre-pregnancy BMI, weight change and cardiac output with individual biomarkers

	Adjusted for age & pre-pregnancy BMI		Adjusted for age, pre-pregnancy BMI, weight change and cardiac output		Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & insulin		Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & glucose		Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & log HOMA		Adjusted for age, pre-pregnancy BMI, weight change, cardiac output & log HS-CRP	
	β (SE)	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**	**β (SE)**	**P value**
Trimester 1	Ref		Ref		Ref		Ref		Ref		Ref	
Trimester 2	**-.595 (.27)***	**.03**	.214 (.30) ‡	.48	.365 (.31)	.25	.509 (.32) †	.11	.374 (.31)	.24	.358 (.29)	.23
Trimester 3	**-.732 (.26)***	**.01**	.846 (.42) †‡	.05	**1.09 (.44)*** †¥	**.02**	**1.29 (.44)*** †¥	**.00**	**1.12 (.44)*** †¥	**.01**	**.951 (.42) *** †¥	**.03**
Postpartum	**-.744 (.27)***	**.01**	**-.735 (.26)***	**.01**	-.541 (.28) †	.06	**-.585 (.29)***	**.049**	-.532 (.28) †	.06	-.320 (.26) †	.23
Age (yr)	.000 (.03)	.997	**-.076 (.03)**	**.02**	**-.078 (.03)**	**.02**	**-.086 (.03)**	**.01**	**-.076 (.03)**	**.02**	**-.082 (.03)**	**.02**
Pre-preg BMI (kg/m^2)	-.086 (.05)	.08	.002 (.05)	.96	-.007 (.05)	.88	-.020 (.05)	.67	-.014 (.05)	.78	-.019 (.05)	.70
Weight change (kg)			**-.074 (.03)**	**.01**	**-.084 (.03)**	**.00**	**-.092 (.03)**	**.00**	**-.089 (.03)**	**.00**	**-.075 (.03)**	**.01**
Cardiac output (L/min)			**-.486 (.10)**	**<.0001**	**-.404 (.11)**	**.0004**	**-.444 (.11)**	**<.0001**	**-.421 (.11)**	**.0002**	**-.490 (.10)**	**<.0001**
Insulin (μU/ml)					-.013 (.02)	.58						
Glucose (mg/dl)							**.028 (.01)**	**.049**				
Log HOMA									.041 (.29)	.89		
Log hsCRP											**.289 (.10)**	**.003**

*Different from first trimester at p<.05. †Different from second trimester at p<.05. ‡Different from postpartum at p <.05. ¥Different from postpartum at p <.01.
Pre-preg BMI is pre-pregnancy body mass index, weight change is from pre-pregnancy, HOMA is homeostasis model assessment, hsCRP is high-sensitivity C-reactive protein.

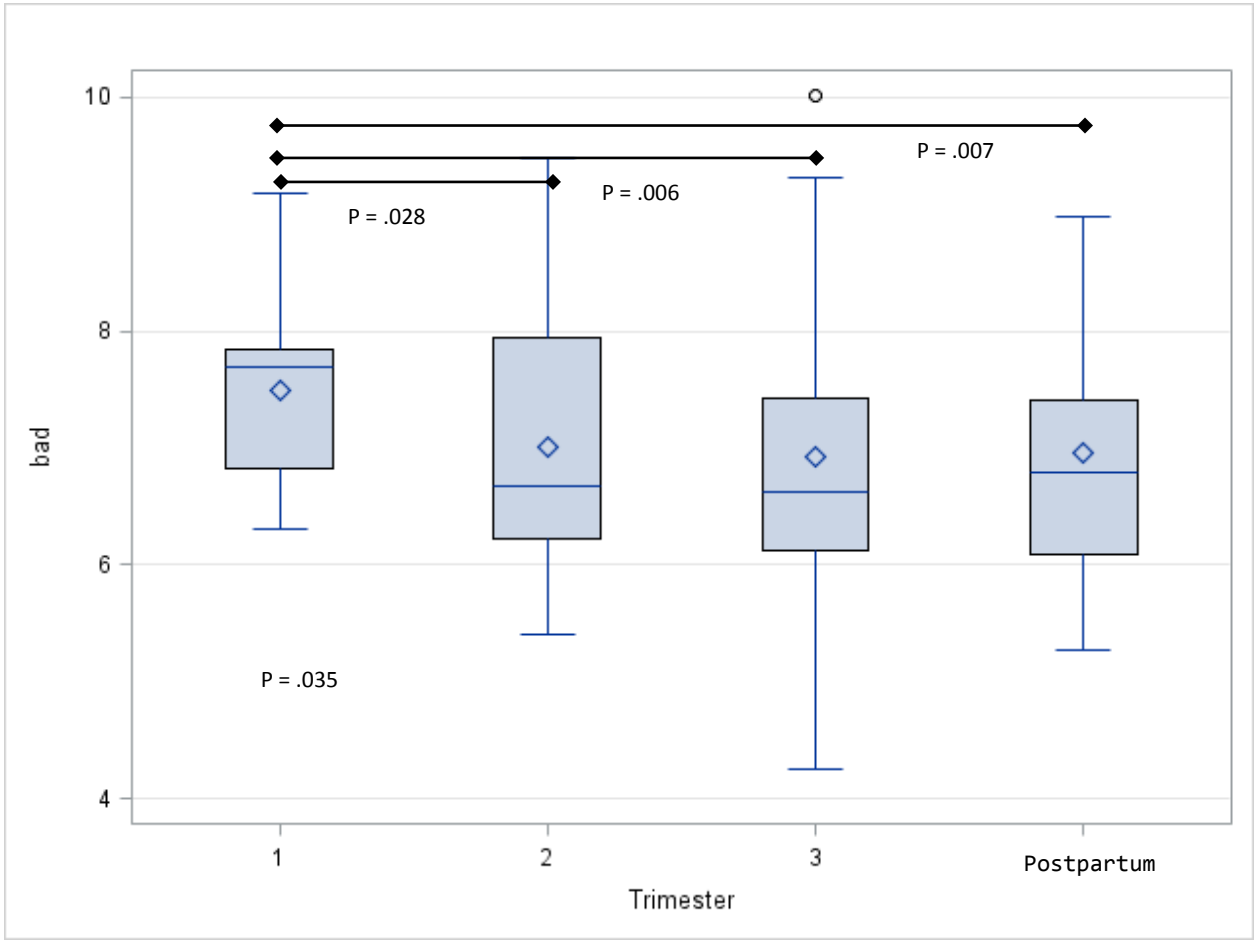

Figure 4.1: Estimated brachial artery distensibility adjusted for age and pre-pregnancy body mass index

5.0 CONCLUSIONS AND PUBLIC HEALTH SIGNFICANCE

5.1 CONCLUSION

The preponderance of epidemiologic studies demonstrate a J-shaped relationship between parity and CVD risk in women, with lowest risk in women with 2 or 3 births, but the reasons for this relationship are not well-understood.[76-78] Elevated risk in women with one or no births might be due to generally poorer health in women who have difficulty bearing children, or due to the fact that pregnancies produce beneficial effects on the cardiovascular system. The elevated risk that occurs with more births could be an example of antagonistic pleiotropy, in which 1 or 2 completed pregnancies have a positive effect on the cardiovascular system but more have a negative effect, or be due to the long term effects from accumulation of CVD risk factors with multiple pregnancies. Additionally, lower SES is associated with higher parity, more CVD risk factors (less healthy lifestyle), and greater CVD risk, and may confound the relationship.[80,212]

This dissertation sheds light on both ends of this relationship. Paper 1 demonstrates that 2 markers of arterial health were worse in nulliparous young, overweight or obese women, than in the parous comparison group. Nulliparous women (n=70, age 34.9 ± 7.1) had higher common carotid IAD (.230 mm, SE .08, P = .006) and mean CCA IMT (.031 mm, SE .01, P = .009) compared with parous women (n=102, age 39.5 ± 4.9) after adjustment for age, race, and CVD risk factors. This difference was similar in magnitude to the difference seen in women with histories of documented infertility in the sample. This suggests that in this sample higher IAD and CCA IMT may be related to undiagnosed fertility problems.

Papers 2 and 3 describe prospective structural and functional changes that occur in large more elastic arteries (carotid artery) and medium-sized more muscular peripheral arteries (brachial artery) throughout the course of the normal first pregnancy and early postpartum, and shed light on the higher CVD risk in women of high parity. These papers found 2 negative vascular changes that occur during pregnancy and persist until at least 8 weeks postpartum: increased CCA IMT and decreased brachial artery distensibility. The increased CCA IMT is not well explained by the atherogenic cardiometabolic changes of pregnancy, and may simply be a response to the increased IAD and hemodynamic changes of pregnancy. The increased stiffness of the brachial artery appears to be explained by the increased weight and cardiac output of pregnancy, consistent with studies of non-pregnant young adults.[209] It has long been thought that pregnancy's acute effects on the vasculature are positive; both systemic vascular resistance and global (combined central and peripheral) arterial stiffness decrease during pregnancy.[88,181] Recent studies, however, suggest that the effects of pregnancy on the vasculature are more mixed. They have found that the carotid artery actually stiffens during the course of pregnancy,[171,194] is stiffer in pregnant women at greater gestational ages,[206] and is stiffer in mid-life and older women of higher parity.[207] Our work supports this new understanding that pregnancy's effects on the vasculature differ by vascular bed. If the increased CCA IMT and brachial artery distensibility persist long term, they may help explain the relationship between high parity and greater CVD risk.

Our research succeeded in illuminating several parts of the conceptual model presented in Figure 2.5. Paper 1, which finds markers of less healthy arteries in nulliparous women comparable to those found in infertile women, suggests that there is a relationship between the health of the systemic vasculature and the ability of women to conceive and carry healthy

pregnancies. Papers 2 and 3 describe prospectively the remodeling of the systemic vasculature during pregnancy, and also demonstrate how those changes persist into the early postpartum period. They explore the effects of physiological changes of pregnancy on these measures. They suggest that the weight gain and increased cardiac output of pregnancy largely explains the increased brachial artery stiffness that we observed, and that the changes in the carotid artery are largely independent of factors traditionally related to atherosclerosis. Reproductive history may provide important information about cardiovascular disease risk independent of traditional CVD risk factors.

There are several areas of future work for this dissertation. The analysis in Paper 1 should be replicated in other samples of women. The largely white, educated sample of overweight and obese young women is not representative of the general US population. It should be replicated in women of healthy weight and more diverse racial and SES backgrounds. If these findings of worsened vascular health in nulliparous women are validated, it could provide an important additional risk factor for identifying women at elevated risk of CVD.

The first step in future research stemming from the MVP study is to assess the women again at 2-3 years after their last study visit, to determine if the negative vascular changes identified as occurring during their first pregnancies persist past the early postpartum period. This study is currently in process, and will provide important information on whether these changes persist. MVP needs to be replicated in a larger sample, and more diverse populations. Ideally, pre-pregnancy measures of vascular, physical and metabolic measures can be obtained so that it can be determined if these affect the changes that occur, as well as what changes occur early in the first trimester of pregnancy. Additionally, this study, which focuses on the normal vascular changes that occur during healthy first pregnancy, has potential to illuminate the many

pregnancy complications that are associated with abnormal vascular remodeling. Cross-sectional studies demonstrate greater IMT and vascular stiffness in women with pregnancy complications such as preeclampsia (See Tables 6.1 and 6.3 in Appendix 1). Prospective studies are needed that include more women who develop pregnancy complications. These could tell us whether women who develop pregnancy complications have less healthy arteries pre-pregnancy or whether they start pregnancy with arteries similar to those of women with healthy pregnancy but vascular remodeling goes awry during the course of pregnancy. If the first is true, research is needed on whether pre-conception risk reduction may decrease incidence of pregnancy complications. If the latter is true, it might provide considerable insight into the pathophysiology of pregnancy complications, and provide early markers for women at risk.

5.2 PUBLIC HEALTH SIGNIFICANCE

Cardiovascular disease is the leading cause of death in women, causing more than 340,000 deaths in 2010.[215] Even small decreases in risk can lead to substantial number of lives saved. Identification of nulliparity as a risk factor for CVD may contribute to the ability of clinicians to efficiently screen for women at elevated CVD risk in order to provide targeted risk reduction efforts to a population at risk. Nulliparity is a risk factor that can be detected in the first half of a woman's life, allowing for an extended period of time for risk reduction to have an effect. While epidemiologic studies have found only about a 10% higher risk of CVD in nulliparous women, as opposed to a 50% higher risk in women of parity of 5 or greater,[77] the proportion of nulliparous women in the population is much higher than the proportion of women

of high parity in the United States,[216] leading to a greater population attributable risk in this group.

This research may also contribute to an understanding of how pregnant women can decrease the negative relationship between parity and CVD. In paper 3 we demonstrate that greater pregnancy weight gain is related to greater stiffening of the brachial artery, a CVD risk factor. Currently, the Institute of Medicine recommends upper limits for healthy pregnancy weight gain, but these are based on maximizing fetal, not maternal, well-being.[185] The committee writing the current recommendations was ”unable to identify any published studies that examined a direct association between GWG (gestational weight gain) and the development of cardiovascular disorders later in life.”[185] If findings that greater gestational weight gain is related to greater arterial stiffness and that this stiffness persists long term, this would provide support for the idea that limiting pregnancy weight gain to recommended levels is healthy not just for the fetus but also for the woman herself.

Paper 2’s description of the normal vascular changes in healthy pregnancy may serve to illuminate some of the most perplexing issues in pregnancy care. Precise causes of preeclampsia, intrauterine growth restriction and preterm birth are not well understood, but there is evidence that many cases of these conditions have vascular components[1-5,52] and that preeclampsia specifically is associated with abnormal adaptation of the carotid artery.[156-158,161,162] Preeclampsia affects 2-8% of US pregnancies[55] and preterm birth 12%.[60] A better understanding of the mechanisms of vascular adaptation to healthy pregnancy may provide a basis for better understanding of the pathophysiology of these pregnancy complications, and perhaps lead to means for early identification of women at risk, or even to prevention.

APPENDIX: Review of Literature

Table A.1 Studies of Intima-Media Thickness and Preeclampsia

Author	Study Design	Population & Sample Size	Definition of Preeclampsia	Outcome Assessed	When Assessed	Confounders	Results
Andersgaard et al. (2012)	Population-based Cross-sectional data Norway	Participants: 2524 total History of preeclampsia: 250 No HTN or proteinuria: 1778 Average age 48 yo Average BMI 25-26	Self-report. "If you have had high blood pressure during pregnancy, was it your first pregnancy?" and "If you have had proteinuria during pregnancy, was it your first pregnancy?" If they replied they had both hypertension and proteinuria, counted as preeclampsia.	IMT – mean of near & far walls of CCA & far wall of bulb.	Average 25 years postpartum	IMT is age-adjusted Women reporting PE had higher MAP, SBP, DBP, total cholesterol, triglycerides, BMI, waist circumference.	Mean IMT 0.86 mm (95% CI 0.84-0.89) for women with PE vs. 0.82 (95% CI 0.81-0.83) for women without HTN or proteinuria (.01>p>.001).
Berends et al. (2008)	Case-control Cross-sectional data Netherlands	Participants: History of PE: 56 Normal pregnancy: 106 Average age: 36 for cases, 39 for controls.	Cases identified from National Birth Registration Records. All records reviewed by author. PE: de novo hypertension (SBP>=140, DBP >=90) & proteinuria >=300mg/24 hours or >=1+ on semiquantitative analysis	CCA IMT bilaterally.	Time from birth: 7 years for cases, 13 years for controls.	Women with PE had higher BMI, larger waist circumference, higher SBP &DBP, higher blood glucose, more frequent metabolic syndrome.	Median IMT 0.66 mm for women with PE vs. 0.68 mm for controls. For women with PE, those with HTN had greater IMT than those without; no difference among controls when stratified by HTN.

Table A.1 Continued Studies of Intima-Media and Preeclampsia

Author	Study Design	Population & Sample Size	Definition of Preeclampsia	Outcome Assessed	When Assessed	Confounders	Results
Blaauw et al. (2006)	Case-control Cross-sectional data Netherlands	Participants: Primiparas with PE: 22 Primiparas normal pregnancy: 22 Nulliparas: 22 Mean BMI 26	Hospitalized at one hospital with early-onset PE (before 34 weeks EGA). New appearance of DBP 90 mm Hg or higher measured at 2 occasions at least 4 hours apart with proteinuria (>=300 mg/24 hours or 2+ dipstick) after 20 weeks. Very strict definition, very high risk group (2 had eclampsia, 73% IUGR).	CCA IMT, Bulb IMT, ICA IMT, common femoral artery IMT, superficial femoral artery IMT bilaterally.	3-13 months postpartum and at least 6 weeks after ending lactation.	Control groups frequency-matched to PE group on age, BMI and smoking. Women with PE had higher triglycerides & homocysteine.	CCA IMT is largest in PE group (0.65), medium in normal pregnancy group (0.62 mm) & least in nulliparous group (0.59). Difference between PE & nulliparous p = .002. After adjustment for BMI, SBP, DBP, HDL, LDL, triglycerides, smoking & family history, this remains the same.
Gaugler-Sender et al. (2008)	Case-control Cross-sectional data Netherlands	Participants: Women with severe early PE: 20 Women with healthy pregnancies: 20 Average age: 32	Severe, early onset preeclampsia (<24 weeks). DBP >= 110 mm HG & proteinuria < 2+ on catheterized specimen, or SBP >= 140 mmHG or DBP >= 90 mmHg on 2 occasional and proteinuria >+ 300mg/24 hours or >=2+ on dipstick of voided specimen with eclampsia or HELLP syndrome.	Mean of maximum IMT of near & far wall of CCA	Mean 5.5 years from index pregnancy, range 4-11.	Controls matched for age, parity, race and year of delivery. There were significant differences in BP, HTN, & microalbuminuria. BMI for cases 25.7 vs. 22.7 for controls, but NS.	Mean Max CCA IMT 0.6 mm for women with PE, 0.59 mm for controls. Only unadjusted results presented.
Goynumer et al. (2013)	Case-control Cross-sectional data Turkey	Participants: Women with severe PE: 34 Women with healthy pregnancies: 42 Average age: 30	Severe preeclampsia: SBP >= 160 mmHg or DBP >= 110 mmHG on 2 occasions at least 6 hours apart on bed rest, or proteinuria >= 5 g/24 hours with HELLP syndrome or eclampsia. Also, women with end-organ involvement.	Mean of 3 images from right and left CCA.	12-24 months postpartum	All non-smokers and mid follicular. Women with PE had greater BMI, SBP, DBP	Mean CCA IMT 0.66 mm for PE, 0.62 for controls, p = 0.025.

Table A.1 Continued Studies of Intima-Media and Preeclampsia

Author	Study Design	Population & Sample Size	Definition of Preeclampsia	Outcome Assessed	When Assessed	Confounders	Results
Yuan et al. (2013)	Case-control Cross-sectional data China	Participants: All nulliparous. Women with "late-PE": 22 Controls: 28 Average age: 29	Based on guidelines of International Society for the Study of Hypertension in Pregnancy. New onset htn after 20 weeks with properly documented proteinuria.	RCCA using MylabTwice high resolution ultrasound using radio frequency signal. IMT & "internal diameter.'	36 weeks EGA	Controls: matched for age & gestational age. Cases were older, but NS (29 vs. 27, p = 0.09) IMT results adjusted for BP & BMI	Mean CCA IMT .46 mm for PE vs. .35 for normal pregnancy, p=0.003. After adjustment for MAP, NS. Internal diameter 7.8 mm for PE vs. 7.2 for controls, p < 0.0001, significant after adjustment for BMI, SBP, DBP, or MAP.

HTN = hypertension; yo = years old; BMI = body mass index; IMT = intima-media thickness; CCA = common carotid artery; PE = preeclampsia; MAP = mean arterial pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; CI = confidence interval; EGA = estimated gestational age; IUGR = intrauterine growth restriction; ICA = internal carotid artery; HDL = high-density lipoprotein; LDL = low-density lipoprotein; HELLP = hemolysis, elevated liver enzymes, low platelets; RCCA = right common carotid artery; NS = non-significant; BP = blood pressure

Table A.2 Studies of Intima-Media Thickness and Gestational Diabetes

Author	Study Design	Population & Sample Size	Definition of GDM	Outcome Assessed	When Assessed	Confounders	Results
Akinci et al. (2008)	Case-control. Cross-sectional data. Turkey	GDM:20 Women with normal GDM screens in previous pregnancy: 19	2 or more abnormal values on 3 hour GTT. Abnormal are ≥95, 180, 155, 140 mg/dl.	IMT – CCA bilaterally.	3 years pp	Age & BMI matched	IMT = 0.56 mm for each (p=0.962)
Akinci et al. (2011)	Case-control Cross-sectional data Turkey	GDM:128 Healthy women experiencing pregnancy in same period with normal GDM screens: 67 Age 35, BMI 28 vs. 24	2 or more abnormal values on 3 hour GTT. Abnormal are ≥ 95, 180, 155, 140 mg/dl.	Mean of CCA, bulb, ICA IMT bilaterally	Mean 3 years pp (range1-7)	Age matched Significant differences in BMI, WC, Glucose, all cholesterol measures, CRP, insulin	Previous GDM: IMT 0.56 mm ±0.08, Controls 0.52 ± 0.06 (p<0.001)
Bayraktar et al. (2012)	Case-control Cross-sectional data Turkey	Former GDM: 81 Insulin: 45 No insulin: 36 Healthy, lean women experiencing pregnancy in same period with normal GDM screens: 35	2 or more abnormal values on 3 hour GTT. Abnormal are ≥ 95, 180, 155, 140 mg/dl. Compared women achieving glycemic control with diet and exercise to women requiring insulin (oral anti-glycemics not an option).	CCA IMT bilaterally.	3 months postpartum	Age matched. Significant differences in BMI, WC, Glucose, all cholesterol measures, fibrinogen, HOMA-IR, CRP	Previous GDM: IMT 0.55 mm ±0.07, Controls 0.52 mm ± 0.05 (p=.014) Insulin: IMT 0.57 mm ± 0.07, no insulin 0.53 mm ± 0.05, control 0.52 ± 0.05. p = 0.007 for insulin vs. control. Carotid IMT positively correlated with BMI, glucose, CRP, IL-6.
Fakhrzadeh et al. (2012)	Case-control Cross-sectional data Iran	Former GDM: 20 Former pregnancy without GDM: 20 Exclude: present or former smokers, HTN, DM, symptomatic CVD	O'Sullivan and Mahan criteria: 2 or more abnormal values on 3 hour GTT. Abnormal are ≥ 105, 190, 165, 145	Mean of 6 measurements: Left and right CCA, bulb & ICA IMT.	4 years postpartum	Matched on BMI, age, and years since pregnancy. Former GDM NS older, higher parity, significantly higher insulin and HOMA-IR	Previous GDM: mean IMT 0.51 mm ± 0.09, Controls 0.48 ± 0.09, p = .02.

Table A.2 Continued Studies of Intima-Media Thickness and Gestational Diabetes

Author	Study Design	Population & Sample Size	Definition of GDM	Outcome Assessed	When Assessed	Confounders	Results
Freire et al. (2012)	Case-control Cross-sectional data Brazil	Former GDM: 79 Former pregnancy without GDM: 60 Exclude: preeclampsia, smoking, addiction, uremia, liver, psychiatric, rheumatological or thyroid disease, or steroid use	2 or more abnormal values on 3 hour GTT. Abnormal are ≥ 95, 180, 155, 140 mg/dl.	Left and right CCA, bulb & ICA IMT. Mean of 6 readings.	2-3 years postpartum	Significant differences is BMI, waist circumference, SBP, DBP, fasting glucose, total cholesterol, HDL-c, LDL-c, triglycerides	Previous GDM vs. Controls (medians): LCCA .53 mm vs. .50 mm, p < .05 L bulb .61 mm vs. .57 mm, p = .001 LICA .45 mm vs. .40 mm, p = .01 RCCA .51 mm vs. .50 mm, NS R bulb .59 mm vs. .55 mm, p < .05 RICA .45 mm vs. .40 mm, p < .05 Composite IMT .53 vs. .49 mm, p < .001 In multivariable analysis age, total cholesterol and prior GDM remained as significant predictors of composite IMT.
Tarim et al. (2006)	Case-control Cross-sectional data Turkey	Women with GDM: 30 Unaffected pregnant women: 40 Excluded: smokers, folic acid or vitamin B12 deficiency, hypertension, multiple gestation, fetal anomalies, diabetes, thyroid disease, family history of CVD	2 or more abnormal values on 3 hour GTT. Abnormal are ≥ 95, 180, 155, 140 mg/dl.	Left common carotid artery IMT.	Mean 25 weeks gestation.	Matched on age and BMI.	GDM.582 ± .066 vs. .543 ± .049, p = .006.
Yousefzadeh et al (2012)	Cohort Iran	50 nulliparous women at high-risk for GDM, 18-35, singleton. Excluded: family history CVD, hypertension, cholesterol lowering medication use, hyperlipidemia, diabetes, renal or liver disease, cancer, recent hormonal medications, smoking, BMI > 35, infertility, PCOS, and plaque > 1.0 mm.	Do not specify diagnostic criteria.	Mean of CCA IMT and ICA IMT bilaterally.	Assessed at 24- 24 weeks and 36-38 weeks gestation.	Women with GDM non-significantly greater BMI & higher blood pressure.	25 women with GDM, 25 without. 2nd trimester: GDM .65 mm ± .07 vs. non-GDM .59 mm ± .06, p = .002. 3rd trimester: GDM .65 mm ± .05 vs. .59 mm ± .04, p < .001.

GDM = gestational diabetes mellitus; GTT = glucose tolerance test; IMT = intima-media thickness; CCA = common carotid artery; pp = postpartum; BMI = body mass index; ICA = internal carotid artery; WC = waist circumference; HOMA-IR = homeostasis model assessment of insulin resistance; CRP = C-reactive protein; IL-6 = interleukin 6; HTN = hypertension; DM = diabetes mellitus; CVD = cardiovascular disease; NS = non-significant; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL-c = high-density lipoprotein concentration; LDL-c = low-density lipoprotein concentration; LCCA = left common carotid artery; L = left; LICA = left internal carotid artery; RCCA = right common carotid artery; R=right; RICA = right internal carotid artery; PCOS = polycystic ovary syndrome

Table A.3 Studies of Arterial Stiffening and Preeclampsia

Author	Study Design	Population & Sample Size	Pregnancy Complication	Outcome Assessed	When Assessed	Confounders	Results
Anastasakis et al. (2008)	Cross-sectional Greece	40 women with healthy singleton pregnancies 34 with normal uterine artery pulsatility 6 developed preeclampsia	Preeclampsia: DBP ≥ 110 mmHg on 1 occasion or DBP ≥ 90 mm Hg on 2 occasions at least 4 hours apart, with urinary protein ≥ 300 mg/24 hours or ≥ 2 by dipstick on 2 consecutive occasions at least 4 hours apart developing after 20 weeks gestation in previously normotensive women.	crPWV	20-23 weeks gestation	No adjustment made. SBP higher in women with PE.	crPWV 9.11 m/s ± 1.26 for normal Doppler vs. 9.08 m/s ± 1.51 for women with preeclampsia, p = .636.
Evans et al. (2011)	Cross-sectional United States	50 women with previous uncomplicated pregnancies. 18 women with pregnancies with preeclampsia	Preeclampsia: SBP ≥ 140 mmHG or DBP ≥ 90 mmHG with proteinuria (≥ 300 mg/24 hours or ≥ 2+ by dipstick on voided sample or ≥+1 on catheterized sample or spot urine protein/creatinine ratio > .3) with hyperuricemia after 20 weeks of pregnancy in previously normotensive women.	Heart-to-brachial PWV	16 months postpartum	DBP and TVR higher in previously preeclamptic women.	Heart-to-brachial PWV 374 cm/s ± 8 for normal vs 405 cm/s ± 20 for women with previous preeclampsia, p = .061.
Kaihura et al. (2009)	Cross-sectional England	69 normotensive pregnant women 54 women with preeclampsia, 7 with preexisting chronic hypertension, 20 early-onset (before 34 weeks.)	Preeclampsia: DBP ≥ 110 mmHg on 1 occasion or DBP ≥ 90 mm Hg on 2 occasions at least 4 hours apart, with urinary protein ≥ 300 mg/24 hours or ≥ 2 by dipstick on 2 consecutive occasions at least 4 hours apart developing after 20 weeks gestation in previously normotensive women.	crPWV	Healthy pregnancies: 31 weeks 4 days ± 36 days Early-onset PE: 28 weeks 4 days ± 23 days Late-onset PE: 36 weeks 3 days ± 12 days	46 women with PE on anti-hypertensive medications. State results similar when women on beta blockers excluded. Different gestational ages. Blacks over-represented in early PE group.	crPWV 9.3 m/s ± 0.7 for early PE, 9.5 m/s ± 1.1 for late PE, and 7.5 m/s ± 1.2 for healthy pregnancy, p =< .0001 for PE vs. healthy, p = .4 for early vs. late PE.

Table A.3 Continued Studies of Arterial Stiffening and Preeclampsia

Author	Study Design	Population & Sample Size	Pregnancy Complication	Outcome Assessed	When Assessed	Confounders	Results
Oyama-Kato et al. (2006)	Longitudinal Japan	183 women 18-39. 16 developed PIH. No multiples, chronic hypertension, hyperlipidemia, diabetes mellitus, smokers.	PIH: SBP ≥ 140 mmHG or DBP ≥ 90 mmHG with or without proteinuria (≥ 300 mg/24 hours or ≥ 2 by dipstick) after 20 weeks of pregnancy in previously normotensive women.	baPWV	Pregnancy: 9-14 weeks, 21-29 weeks, 32-39 weeks. Postpartum: 3-5 days, 1 month.	Women in PIH group were older and had greater BMI.	Women in both groups had equivalent baPWV in 1st trimester. For women with healthy pregnancies, this dropped in 2nd trimester and remained decreased 3rd trimester. For women with PIH there was no 2nd trimester drop. Values were significantly higher for women with PIH in 2nd & 3rd trimesters and immediately postpartum.
Robb et al (2009)	Longitudinal Scotland	22 healthy, nulliparous, non-smoking women. No hypertension, medication use, pregnancy complications. 15 women with preeclampsia.	Preeclampsia: SBP ≥ 140 mmHG or DBP ≥ 90 mmHG with proteinuria (≥ 300 mg/24 hours or ≥ 1 by dipstick or spot urine protein/creatinine ratio > 30 mg protein/mmol creatinine) after 20 weeks of pregnancy in previously normotensive women.	crPWV	Normal: 16, 24, 32 & 37 weeks of pregnancy & 7 week postpartum Preeclampsia: after diagnosis & 7 weeks postpartum	Women with preeclampsia were shorter. 7 women in preeclampsia group taking anti-hypertensive medications, 6 received bethamethasone	crPWV was higher in women with preeclampsia when matched to women with normal pregnancy by gestational age (p≤.006). No difference by 7 weeks postpartum.
Spaanderman et al. (2000)	Longitudinal The Netherlands	42 women with history of preeclampsia 10 healthy parous controls No: vitamins, oral contraceptives, blood pressure medications, NSAIDS or glucocorticoids	Preeclampsia: ISSHP guidelines	Femoral artery compliance measured with B-mode ultrasound. Distensibility coefficient.	Pre-pregnancy, 5 & 7 weeks of pregnancy		No difference at 5 week. At 7 weeks women with preeclampsia but no underlying disorder had lower distensibility (6.1 kPa^{-1} ± 2.0 vs. 10.6 kPA^{-1} ± 4.1, p<.05).

Table A.3 Continued Studies of Arterial Stiffening and Preeclampsia

Author	Study Design	Population & Sample Size	Pregnancy Complication	Outcome Assessed	When Assessed	Confounders	Results
Spaanderman et al. (2005)	Longitudinal The Netherlands	31 women with history of preeclampsia, currently normotensive, achieving pregnancy within 1 year. Non-smokers.	Preeclampsia: SBP ≥ 140 mmHG or DBP ≥ 90 mmHG with proteinuria (≥ 300 mg/24 hours) after 20 weeks of pregnancy in previously normotensive women. SGA: birth weight below 10th percentile	Femoral artery compliance measured with B-mode ultrasound. Distensibility coefficient.	Follicular phase of menstrual cycle. 2.3 years since pregnancy.		Femoral artery distensibility: AGA: 6.4 kPa^{-1} (2.7-11.1) SGA: 8.8 kPa^{-1} (5.1-17.0), p <.05.

DBP = diastolic blood pressure; crPWV = carotid-radial pulse wave velocity; DBP = diastolic blood pressure; PE = preeclampsia; SBP = systolic blood pressure; PWV = pulse wave velocity; TVR = total vascular resistance; PIH = pregnancy-induced hypertension; baPWV = brachial-ankle pulse wave velocity; BMI = body mass index; SGA= small for gestation age; AGA = appropriate for gestational age.

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