Abstract

Prospective epidemiological studies indicate that regular exercise during the year prior to conception reduces preeclampsia risk, whereas exercise during affects pregnancy reduces preeclampsia risk only at specific dosages, or in specific subpopulations. The risk of severe preeclampsia is increased among women who exercise for more than 270 minutes/week in early pregnancy. Physiology studies are needed to identify mechanisms through which regular exercise may influence preeclampsia risk. This dissertation examined the effects of pregnancy (30-36 weeks gestation), and regular exercise participation, on two important pathophysiological features of preeclampsia; circulating anti-angiogenic markers, represented by soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), and endothelial dysfunction. The results demonstrate that regularly exercising, pregnant non-smoking women have higher levels of serum placental growth factor (PIGF), lower levels of serum sFlt-1 and sFlt-1:PIGF, and are less likely to experience high serum sEng levels, than sedentary women. The effects of exercise on PIGF and sFlt-1:PIGF are more pronounced among women exercising less than 270 minutes/week in pregnancy. Anti-angiogenic changes that could contribute to preeclampsia were not observed immediately after short-duration, moderate-intensity exercise in the third trimester. Flow-mediated dilation (FMD) and the shear stimulus for FMD are not affected by pregnancy, however the time to peak FMD was increased in pregnancy. Regular exercise did not affect FMD or its shear stimulus in healthy pregnant or non-pregnant women. FMD and its shear stimulus were positively correlated in active, but not inactive, pregnant and non-pregnant women. Pregnancy and physical activity do not affect radial artery low flow-mediated constriction (L-FMC). L-FMC is artery dependent, occurring in the radial, but not the brachial, artery of healthy pregnant and non-pregnant women. The positive correlation between L-FMC and FMD suggests that L-FMC and FMD are not independent measurements. The results of this thesis suggest that physical
activity and exercise may reduce preeclampsia risk by reducing concentrations of angiogenic markers. Although exercise participation did not affect conduit artery vascular function in healthy pregnant women, future studies should investigate the effects of exercise on vascular function in women with endothelial dysfunction, or risk factors for preeclampsia.
Co-Authorship

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Chapter 1
Introduction

Preeclampsia is a serious maternal-fetal disease that affects 2-7% of pregnancies in healthy nulliparous women,\(^1,2\) and is diagnosed after 20 weeks gestation on the basis of new onset hypertension (blood pressure > 140/90 mm Hg for two consecutive readings)\(^3\) and proteinuria (24 hour urinary protein level of at least 0.3 g/day).\(^3\) Preeclampsia should also be suspected without proteinuria if the patient presents with new onset hypertension and other symptoms of major organ dysfunction including thrombocytopenia, elevated liver enzyme activities, persistent headaches, visual disturbances, or epigastric pain.\(^4,5\)

A diagnosis of preeclampsia can have serious implications for both mother and fetus. Preeclampsia accounts for 15% of pre-term births and their associated morbidities and mortality,\(^6\) and can also lead to intrauterine growth restriction and death.\(^7\) Maternal complications include an increased risk of abruptio placentae, renal failure, pulmonary edema, cerebral hemorrhage, stroke and circulatory collapse.\(^7,8\) Careful management has led to a decrease in maternal mortality resulting from preeclampsia in developed countries,\(^9\) however the associated maternal mortality rate in the developing world remains high.\(^10\)

Interest in the relationship between preeclampsia and physical activity was recently ignited by retrospective case-control studies indicating that women who exercised regularly were 25 to 60% less likely to develop preeclampsia than sedentary women.\(^11-15\) Three prospective epidemiological studies, however, produced conflicting results.\(^16-18\) In 2,241 American women, any activity during the year prior to conception was associated with a 45% reduction in preeclampsia risk (0.55 (odds ratio (OR)); 0.30-1.02 (95% confidence interval)).\(^19\) Similar relationships were observed when
Physical activity was quantified by minutes/week, energy expenditure, and rating of perceived exertion (RPE). In 59,573 Norwegian women, however, preeclampsia risk was not reduced among women who performed any exercise during early pregnancy, compared to sedentary controls (0.94; 0.85-1.04). Physical activity participation during early pregnancy also had no impact on the risk of developing all subtypes of preeclampsia in 85,139 Danish women. The risk of severe preeclampsia was increased among women exercising ≥270 min/wk, compared with sedentary women (OR 1.65-1.78, depending on weekly physical activity duration).

While the two largest studies did not observe a protective effect of exercise during early pregnancy in the entire sample, protective effects were reported in specific subpopulations or at specific dosages. Preeclampsia risk was reduced in Norwegian women who exercised ≥25 times/month during early pregnancy (0.79; 0.65-0.96). Among Danish women who were not exposed to smoke during pregnancy, women who exercised less than 270 minutes/week had a reduced risk of severe preeclampsia (adjusted ORs 0.43-0.59), whereas women who exercised more than 270 minutes/week had an increased risk of severe preeclampsia (adjusted ORs 2.35-3.48).

Studies reporting a protective effect of exercise against preeclampsia in all women, in specific subpopulations or at specific dosages, have led researchers to hypothesize that exercise may reduce preeclampsia risk by enhancing placental growth and development, reducing oxidative stress, preventing immune maladaptation, and correcting endothelial dysfunction. Researchers have also hypothesized that acute exercise may exacerbate the pathophysiological processes that lead to preeclampsia, explaining the increased in the risk of severe preeclampsia among women who exercised more than 270 minutes/week. Hypothesized mechanisms concerning the relationship between exercise and preeclampsia risk are primarily based on data
collected in men and non-pregnant women, or in patients with other conditions characterized by endothelial dysfunction.\textsuperscript{20, 21} Few studies have examined the effects of exercise on pathophysiological processes involved in the development of preeclampsia in pregnant women with no overt risk factors, and women with risk factors for preeclampsia. Physiology studies examining the mechanisms through which regular exercise may influence preeclampsia risk in pregnant women are therefore needed.

Two important pathophysiological features of preeclampsia are excessive increases in the circulating anti-angiogenic markers soluble fms-like tyrosine kinase-1 (sFlt-1)\textsuperscript{22} and soluble endoglin (sEng),\textsuperscript{23} and endothelial dysfunction.\textsuperscript{24} sFlt-1 is the one of two receptors for placental growth factor (PIGF) and one of three receptors for vascular endothelial growth factor (VEGF).\textsuperscript{25} sFlt-1 reduces angiogenesis by binding these ligands as a non-signaling decoy,\textsuperscript{25} and causes hypertension, proteinuria, and glomerular endotheliosis in rats.\textsuperscript{22} Serum sFlt-1 is elevated five-fold in women with preeclampsia, compared with normotensive pregnant women.\textsuperscript{22} These anti-angiogenic effects of sFlt-1 may impair pregnancy-induced adaptation in maternal myometrial vessels, and contribute to systemic maternal endothelial dysfunction.\textsuperscript{25} sEng is the extra-cellular domain of Endoglin (Eng), a co-receptor for transforming growth factor (TGF) $\beta 1$ and $\beta 3$. sEng inhibits binding of TGF $\beta 1$ to Eng, preventing endothelial nitric oxide synthase activation and subsequent vasodilation.\textsuperscript{23} Combined sEng and sFlt-1 overexpression in rats causes symptoms that mimic severe preeclampsia, including proteinuria, severe hypertension, intrauterine growth restriction, and low platelet counts.\textsuperscript{23} Serum sEng concentrations are elevated three-fold in women with mild preeclampsia, and five-fold in women with severe preeclampsia, compared to normotensive pregnant women.\textsuperscript{21} No study has assessed the effects of regular exercise on sFlt-1 and sEng in non-pregnant, pregnant, or preeclamptic, women. This information is essential to determine whether any effect of regular exercise on preeclampsia risk may be mediated through
anti-angiogenic molecules. Researchers have also hypothesized that an acute exercise bout may exacerbate the pathophysiological processes that lead to preeclampsia, providing a mechanism by which repeated bouts of acute exercise could increase the risk of severe preeclampsia among women exercising more than 270 minutes/week in early pregnancy. Studies examining the effects of acute exercise on anti-angiogenic markers during pregnancy are therefore needed to test the hypothesis that acute exercise has anti-angiogenic effects that may increase the risk of preeclampsia.

Endothelial dysfunction is a second critical feature of preeclampsia, which leads to many late stage symptoms of the syndrome. Conduit artery endothelial function is frequently assessed using brachial artery flow-mediated dilation (FMD), a non-invasive measurement of endothelium-dependent dilation in response to increased shear stress. Decreased FMD predicts cardiovascular events in a variety of populations, and is observed prior to, during, and following preeclampsia. The only study which examined the effects of physical activity on brachial artery FMD in young women did not include pregnant subjects, and observed no effect of regular exercise on FMD in the early follicular phase. FMD was measured distal to the occlusion cuff, and therefore ischemia and decreases in transmural pressure during occlusion, and increased shear stress following cuff release, may all have contributed to the observed dilation. The effects of pregnancy on FMD have not been conclusively determined. Studies examining the effects of pregnancy on FMD have observed increases, or no change, at 28 to 36 weeks gestation compared to non-pregnant controls. These studies have three important limitations which may have contributed to the lack of consensus. First, studies measured post-release dilation at a pre-defined time point, rather than using continuous measurements to identify true peak FMD. This approach does not account for inter-individual or between-group differences in the time of peak dilation, and conclusions may be misleading if pregnancy
systematically alters the timing of peak FMD. Second, existing studies did not test non-pregnant controls in the mid-late luteal phase of the menstrual cycle, which is the phase most similar to pregnancy.\textsuperscript{44,45} FMD varies during the menstrual cycle,\textsuperscript{46,47} and differences between pregnant and non-pregnant women\textsuperscript{36,37} may therefore be due to the inclusion of women in multiple menstrual cycle phases rather than to pregnancy. Third, existing studies did not account for physical activity, or potential pregnancy-induced changes in the relevant shear stimulus for FMD. Research is needed to address these limitations, and to evaluate the effects of pregnancy and physical activity on brachial artery FMD.

The degree of radial artery vasoconstriction during reduced flow caused by wrist occlusion (low flow-mediated constriction, L-FMC) has recently been proposed as a companion vascular function test to FMD.\textsuperscript{48} Radial artery L-FMC may be mediated through a combination of endothelin-1, cyclooxygenase products, and an endothelium-derived hyperpolarizing factor,\textsuperscript{48-50} and is diminished in hypertensive patients compared to healthy, young subjects.\textsuperscript{48}

Recommendations that L-FMC be measured concurrently with FMD were based on radial artery data,\textsuperscript{48} however, the brachial artery is traditionally used for FMD assessment.\textsuperscript{27-29,51} Before L-FMC can be used to test vascular function in clinical populations, including pregnant and preeclamptic women, it is important to determine whether L-FMC occurs in the brachial artery. The effect of artery location on L-FMC has not been systematically examined, however, available data suggest that L-FMC in healthy subjects may be artery dependant. Three studies reported mean radial artery L-FMC values of 4-7\% among healthy young and middle aged subjects during 4.5-5 minutes of wrist occlusion.\textsuperscript{48,50,52} Results in the brachial artery are inconsistent, as investigators observed changes in mean brachial artery diameter of 2.4\%,\textsuperscript{53} -1.1\%,\textsuperscript{26} -1.7\%,\textsuperscript{54} and
-15%\textsuperscript{55} during 5 minutes of forearm occlusion in healthy young men and women. In middle-aged men and women, brachial artery diameter did not change during 5 minutes of forearm occlusion in healthy non-smokers,\textsuperscript{56} or during 5 minutes of wrist occlusion in normocholesterolemic control subjects.\textsuperscript{57} These results\textsuperscript{26, 53-58} suggest that L-FMC occurs in the radial, but not the brachial, artery in healthy subjects, however, paired measurements are required to confirm this hypothesis.

Previous studies also proposed that the sum of the absolute values of L-FMC and FMD may provide a more complete assessment of vascular function by incorporating responsiveness to both decreases and increases in shear stress from baseline levels.\textsuperscript{48} This interpretation of L-FMC and FMD as separate indicators is based on the assumption that L-FMC has fully reversed before peak FMD occurs. This assumption has not been tested. If the factors regulating L-FMC do not return to pre-inflation values before peak FMD, then FMD may be a composite of hyperemia-induced dilation, superimposed on the return to baseline of factors regulating L-FMC following removal of the low-shear stimulus. There was no relationship between radial artery L-FMC and FMD in a previous study, supporting the conclusion that L-FMC and FMD provide independent information about endothelial function.\textsuperscript{48} Additional studies are needed to confirm this finding, and to examine the relationship between L-FMC and FMD in diverse populations, including pregnant and non-pregnant women.

1.1 Objectives and Hypotheses

**Objective 1 (Chapter 3):** To determine whether serum sEng, VEGF, sFlt-1, PlGF and sFlt-1:PlGF differ between active and inactive women in late gestation. **Hypothesis 1:** Serum VEGF,
PIGF, sFlt-1, sFlt-1:PIGF and sEng do not differ between active and inactive pregnant women at 30-36 weeks gestation.

**Objective 2 (Chapter 3):** To assess the effects of acute, moderate-intensity exercise on serum sEng, sFlt-1, VEGF, and PIGF in pregnant and non-pregnant women. **Hypothesis 2:** Acute exercise does not affect serum sEng, sFlt-1, VEGF, or PIGF in pregnant or non-pregnant women.

**Objective 3 (Chapter 4):** To determine whether pregnancy-induced increases in brachial artery diameter are due to reduced vascular tone secondary to increased shear-mediated dilation. **Hypothesis 3:** Pre-inflation brachial artery diameter and shear rate are increased in pregnant women compared to non-pregnant controls. Removal of the baseline shear stimulus during 5 minutes of distal cuff occlusion does not significantly change artery diameter in pregnant women, indicating that shear-mediated dilation does not increase resting brachial artery diameter in the third trimester.

**Objective 4 (Chapter 4):** To assess the effects of pregnancy and physical activity on true peak brachial artery FMD by using continuous measurements to account for inter-individual and between-group differences in the timing of peak dilation. **Hypothesis 4:** True peak brachial artery FMD is not different in non-pregnant women in the mid-late luteal phase, and pregnant women at 30-36 weeks gestation. Physical activity has no effect on FMD in pregnant and non-pregnant women.
Objective 5 (Chapter 5): To examine the effects of pregnancy and activity on L-FMC.

Hypothesis 5: L-FMC is not different between pregnant and non-pregnant, or between active and inactive, women.

Objective 6 (Chapter 5): To determine whether L-FMC is artery-specific in healthy pregnant and non-pregnant women. Hypothesis 6: L-FMC occurs in the radial artery, but does not occur in the brachial artery, of healthy pregnant and non-pregnant women.

Objective 7 (Chapter 5): To assess the relationship between L-FMC and FMD, and to determine whether pregnancy or physical activity modify the relationship between L-FMC and FMD.

Hypothesis 7: L-FMC is not correlated with FMD in the radial artery of active and inactive, pregnant and non-pregnant women. This indicates that the two measurements provide independent information about endothelial function.
1.2 References


Chapter 2
Physiological Mechanisms Which Could Effect the Relationship Between Regular Physical Activity and Preeclampsia Risk

2.1 Abstract
Preeclampsia is a serious maternal-fetal disease that affects 2-7% of pregnancies.\textsuperscript{1,2} The etiology of preeclampsia likely involves an interaction between impaired placental development, maternal constitutional factors, genetic susceptibility, inflammation, oxidative stress, and endothelial dysfunction.\textsuperscript{3,4} Regular exercise during the year prior to conception may protect against preeclampsia,\textsuperscript{5} whereas regular exercise during early pregnancy does not alter preeclampsia risk in general,\textsuperscript{5-7} but may reduce preeclampsia risk in specific subpopulations\textsuperscript{7} or at specific dosages.\textsuperscript{6,7} The risk of severe preeclampsia is increased among women who exercise more than 270 minutes/week in early pregnancy, compared to sedentary controls.\textsuperscript{7} Physiological studies are needed to test two hypotheses concerning the relationship between preeclampsia and exercise. The first proposes that regular exercise prior to pregnancy, or during early pregnancy in specific subpopulations, may prevent preeclampsia by enhancing placental vascular growth, reducing inflammation, increasing the concentration and activity of antioxidants to reduce oxidative stress, and correcting endothelial dysfunction.\textsuperscript{3,4} The second hypothesis that the acute effects of exercise may increase the risk of severe preeclampsia by exacerbating pathophysiological processes associated with preeclampsia.\textsuperscript{7} As new guidelines for management of mild term preeclampsia are shifting away from bedrest and towards outpatient monitoring,\textsuperscript{8,9} physical activity and exercise safety in women with gestational hypertension and mild term preeclampsia should be assessed.

\textbf{Keywords:} preeclampsia, exercise, oxidative stress, endothelial function, angiogenesis
2.2 Introduction

Preeclampsia is a serious maternal-fetal disease that affects 2-7% of pregnancies,¹,² and is diagnosed after 20 weeks gestation on the basis of new onset hypertension (blood pressure > 140/90 mm Hg for two consecutive readings)¹⁰ and proteinuria (24 hour urinary protein level of at least 0.3 g/day).¹⁰ Preeclampsia should also be suspected without proteinuria if the patient presents with new onset hypertension and other symptoms of major organ dysfunction including thrombocytopenia, elevated liver enzyme activities, persistent headaches, visual disturbances, or epigastric pain.¹¹,¹²

A diagnosis of preeclampsia can have serious implications for both mother and fetus. Preeclampsia accounts for 15% of pre-term births and their associated morbidities and mortality,¹³ and can also lead to intrauterine growth restriction and death.¹⁴ Maternal complications include an increased risk of abruptio placentae, renal failure, pulmonary edema, cerebral hemorrhage, stroke and circulatory collapse.¹⁴,¹⁵ Careful management has led to a decrease in maternal mortality resulting from preeclampsia in developed countries,¹⁶ however, the associated maternal mortality rate in the developing world remains high.¹⁷

Preeclampsia is often called the “disease of theories”, as many factors are believed to contribute to the pathophysiology of preeclampsia.¹⁴ These include abnormal placental development, predisposing maternal constitutional factors, oxidative stress, immune maladaptation, and genetic susceptibility.¹⁸-²⁰ Each of these factors contributes to systemic maternal endothelial dysfunction, which leads to vasoconstriction and reduced perfusion of critical organs and tissues. While the
pathophysiological processes that lead to preeclampsia begin in early pregnancy, maternal symptoms do not appear until mid to late gestation. The severity of symptoms can accelerate rapidly, leading to life-threatening seizures (eclampsia) and the necessity for immediate delivery regardless of gestational age.

Other than delivery, there are no proven interventions to treat preeclampsia or to prolong gestation. However, recent attention has focused on the potential benefits of regular exercise in preventing preeclampsia. Retrospective case-control studies indicate that women who exercise regularly are 25-60% less likely to develop preeclampsia, while a prospective epidemiological study observed a 45% reduction in preeclampsia risk among women who participated in any regular exercise during the year prior to conception. However, two additional prospective epidemiological studies reported that participation in any regular exercise during early pregnancy did not alter preeclampsia risk in general, but may affect preeclampsia risk in specific subpopulations or at specific dosages. The first study reported that preeclampsia risk was reduced among women who exercised more than 25 times/month in early pregnancy. The second study observed a reduced risk of severe preeclampsia among women who were unexposed to smoke and exercised less than 270 minutes/week in early pregnancy, compared to sedentary women. However the risk of severe preeclampsia was increased among women who exercised more than 270 minutes/week in early pregnancy, when compared to sedentary controls. These results suggest that the effect of exercise on preeclampsia risk depends on the timing of the exercise with respect to the index pregnancy, the strength of the exercise stimulus, the subtype of preeclampsia, and the underlying characteristics of the subject population.

Research examining the physiological mechanisms by which regular exercise could decrease the risk of preeclampsia, or increase the risk of severe preeclampsia, is urgently needed. After briefly
describing current theories on the pathophysiology of preeclampsia, this review will focus on four key objectives. First, the epidemiological evidence that exercise affects preeclampsia risk will be reviewed. Second, evidence will be discussed as a basis for proposing that regular physical activity prior to conception, or in early pregnancy among women in specific subpopulations or at specific dosages, may protect against preeclampsia by intervening at four key stages in the disease process: (1) enhancing placental growth and vascularity (protection against abnormal placental development), (2) decreasing oxidative stress, (3) reducing inflammation, and (4) reversing endothelial dysfunction. Third, mechanisms by which acute exercise may increase the risk of severe preeclampsia will be examined. Fourth, as new guidelines for management of mild term preeclampsia are shifting away from bedrest and towards outpatient monitoring, the importance of assessing physical activity and exercise safety in women with gestational hypertension and mild term preeclampsia will be discussed.

2.3 Etiology

Hypothetical underlying causes of preeclampsia can be divided into five main categories: abnormal placental development, predisposing maternal constitutional factors, oxidative stress, immune maladaptation, and genetic susceptibility. Each of these hypothetical causes contributes to endothelial dysfunction, which then leads to late-stage symptoms of preeclampsia. These hypothetical causes are not mutually exclusive, and probably interact to produce the symptoms associated with preeclampsia. The proposed mechanisms may also be sequential events in the pathogenesis of preeclampsia. Abnormal placental development and predisposing maternal constitutional factors, for example, both cause oxidative stress, which would contribute to the development of endothelial dysfunction and preeclampsia. Each of these five theories has been reviewed extensively elsewhere, and will be described briefly here.
**Abnormal Placental Development:** During normal pregnancy, extravillous trophoblasts invade down the lumens of the uterine spiral arteries and between the uterine glands. Replacement of maternal endothelial and vascular smooth muscle cells with fetal trophoblast transforms the uterine spiral arteries from narrow, high resistance vessels to wide, low-resistance conduits that transport blood into the intervillous space. Trophoblast invasion is initiated at the center of the placenta and progresses towards the periphery, such that invasion is deeper in the central regions. Plugs of invading endovascular trophoblasts occlude the uterine spiral arteries in early pregnancy, stopping maternal blood from entering the intervillous space. This low oxygen environment may prevent free radical damage to fetal tissues during early development. The trophoblastic plugs begin to dissipate at 8-9 weeks gestation, allowing limited blood flow into the intervillous space. This process is initiated in the periphery of the placenta, where trophoblast invasion and plugging of the uterine spiral arteries is less complete, and moves towards the center. The antioxidant capacity of the syncytiotrophoblast is limited when maternal blood first enters the intervillous space, and the increased concentration of reactive oxygen species (ROS) that accompanies maternal blood flow leads to villous tissue damage and regression in the periphery of the placenta. Antioxidant capacity increases in parallel with the onset of blood flow, such that villous tissue in the center of the placenta is preserved, and mediates exchange between the maternal and fetal circulations throughout the remainder of pregnancy.

Among women who later develop preeclampsia, trophoblastic invasion of the uterine spiral arteries is usually incomplete. Vessels are narrower than those observed in normal pregnancies, and portions of the maternal endothelial and smooth muscle cell layer remain intact and may be capable of responding to maternal vasoconstrictors. Shallow trophoblastic invasion may cause persistent placental hypoxia or repeated episodes of hypoxia and reperfusion. The underperfused placenta may produce toxins, such as cytokines, lipid peroxides, or elevated
concentrations of placental villous tissue fragments. When released into the maternal circulation, these substances could contribute to oxidative stress and systemic endothelial dysfunction. The risk of preeclampsia is 3.5 times greater in twin pregnancies than in singleton pregnancies, suggesting that the larger placental mass releases additional toxins into the maternal circulation. Recent research has focused on placental release of soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble Endoglin (sEng). sFlt-1 is one of two receptors for PI GF and one of three receptors for VEGF. sFlt-1 serves as a non-signaling decoy when binding these ligands and therefore reduces angiogenesis, causing a preeclampsia-like syndrome characterized by hypertension, protenuiria, and glomerular endotheliosis in mice. Serum sFlt-1 is increased approximately fivefold in women with preeclampsia compared with normotensive pregnant women, and decreases in serum free vascular endothelial growth factor (VEGF) and placental growth factor (PI GF) in preclamptic women are proportional to increases in sFlt-1. Decreased free VEGF and PI GF concentrations due to binding with sFlt-1 may impair normal pregnancy-induced vascular adaptation in maternal myometrial vessels, and contribute to systemic maternal endothelial dysfunction. sEng is the extra-cellular domain of Endoglin (Eng), a co-receptor for transforming growth factor (TGF) β1 and β3. sEng inhibits binding of TGF β1 to Eng, preventing eNOS activation and subsequent vasodilation. Combined sEng and sFlt-1 overexpression in rats causes symptoms of severe preeclampsia, including proteinuria, severe hypertension, intrauterine growth restriction, and low platelet counts. Compared to normotensive controls, sEng concentrations in serum from preeclamptic women are increased three-fold in women with mild preeclampsia and five-fold in women with severe preeclampsia.

**Predisposing Maternal Constitutional Factors:** Several maternal constitutional factors that increase the risk of preeclampsia (diabetes, hypertension, obesity, hyperlipidemia) are also risk factors for atherosclerosis. These conditions may cause oxidative stress and endothelial
dysfunction prior to pregnancy, increasing maternal susceptibility to preeclampsia. Recent research has focused on the angiotensin receptor 1 autoantibodies (AT1-AAs), which are present in serum from preeclamptic women and stimulate the AT1 receptor by binding to its second extracellular loop. AT1-AAs are not present in sera from normotensive pregnant women, and concentrations decrease post-partum in women who developed preeclampsia. Pregnant mice injected with immunoglobulin G (IgG) from preeclamptic women demonstrate many symptoms of preeclampsia, including renal endothelial damage, increased urinary protein and blood pressure, and decreased placental size and fetal weight. These symptoms are strongly attenuated or eliminated by blocking the AT1 receptor, and do not occur in mice injected with IgG from normotensive pregnant women. Injection of IgG from women with preeclampsia increases concentrations of sFlt-1 and sEng in pregnant mice. These results indicate that AT1 receptor activation by AT1-AAs may cause many symptoms of preeclampsia, in part by increasing placental release of sFlt-1 and sEng.

**Oxidative Stress:** Excessive pro-oxidant accumulation may contribute to systemic endothelial dysfunction in preeclamptic women. Oxidative protein damage and lipid peroxidation, indicated by concentrations of F2-isoprostanes, and malondialdehyde and 1-methyl-2(E)-noenal, are increased in the placentas of preeclamptic women, compared to those of normotensive controls. The activities of anti-oxidant enzymes, including super-oxide dismutase, glutathione peroxidase, and thioredoxin reductase, are also reduced in the placentas of preeclamptic women at delivery, compared to normotensive women matched for gestational age. Evidence for systemic maternal oxidative stress in preeclampsia includes increased plasma biomarkers of lipid peroxidation, including F2-isoprostanes, and malondialdehyde, and decreased plasma glutathione concentrations, compared to normotensive controls. The underlying causes of oxidative stress are believed to include abnormal placental development and predisposing
maternal constitutional factors.\textsuperscript{19} It is therefore unclear whether oxidative stress is a stage in the disease process or a distinct cause of preeclampsia.

**Immune Maladaptation:** Paternal genetic material in the fetal trophoblast may activate the maternal immune system, triggering a widespread nonspecific inflammatory response.\textsuperscript{20} The endothelial activation and dysfunction observed in preeclampsia are consistent with vascular inflammation.\textsuperscript{20} Recent research has focused on the relationship between the human leukocyte antigen C (HLA-C) allotype of fetal extravillous trophoblast, and maternal killer immunoglobulin receptors (KIRs) on uterine natural killer (uNK) cells.\textsuperscript{42} Individuals with the AA KIR genotype typically lack activating KIRs, whereas individuals with the AB or BB genotypes possess both activating and inhibitory receptors.\textsuperscript{42} The inhibitory AA genotype occurred in 35\% of women who developed preeclampsia, compared with only 25\% of controls.\textsuperscript{42} When acting as ligands for KIRs, HLA-C can be subdivided into C1 and C2 epitopes. The AA genotype occurred more frequently among women who developed preeclampsia only when combined with the HLA-C2 allotype on fetal trophoblast.\textsuperscript{42} In mothers with the inhibitory AA genotype, binding of trophoblastic HLA-C2 to uNK KIRs may impair trophoblastic invasion through excessive inhibition of uNK cells.\textsuperscript{42} AA genotype frequency did not differ between women with preeclampsia and controls when fetal extravillous trophoblast expressed the HLA-C1 allotype, suggesting that HLA-C1 inhibits uNK cells to a lesser extent than HLA-C2.\textsuperscript{42}

**Genetic Susceptibility:** Women who were born from a preeclamptic pregnancy are twice as likely to develop preeclampsia, and three times more likely to develop early onset preeclampsia, as women who were born from a pregnancy unaffected by preeclampsia.\textsuperscript{43} This two-fold increase in preeclampsia risk is also observed among women born from a pregnancy that was not affected by preeclampsia, who have a sister who was born from a preeclamptic pregnancy.\textsuperscript{43} These results
indicate that maternal genes contribute to preeclampsia risk, and that the genetic component of preeclampsia risk is stronger for severe, early onset preeclampsia than for mild, term preeclampsia.\textsuperscript{43, 44} Men who were born from a preeclamptic pregnancy are 1.5 times more likely to father a preeclamptic pregnancy than men who were born from an unaffected pregnancy.\textsuperscript{43} The risk of fathering a preeclamptic pregnancy is not increased among brothers of men born after a preeclamptic pregnancy, who were born from a pregnancy that was not effected by preeclampsia.\textsuperscript{43} Therefore, placentally-expressed paternal genes also modify preeclampsia risk,\textsuperscript{43} possibly by altering the maternal phenotype.\textsuperscript{44} Genetic studies in families with a history of recurrent preeclampsia suggest that candidate genes which contribute to an increased risk of preeclampsia differ depending on the subtype of preeclampsia, and population studied.\textsuperscript{44, 45} Genes affecting early placental development may be involved in all forms of preeclampsia, whereas genes affecting maternal constitutional factors may be more important in mild term preeclampsia.\textsuperscript{44} Research to identify specific maternal and placental genes that may cause severe, early onset, and mild term preeclampsia in diverse populations is ongoing.\textsuperscript{44, 45}

Multiple underlying causes may explain the heterogeneity of patient symptoms. Preeclampsia, for example, is associated with an increased incidence of both small\textsuperscript{46} and large for gestational age infants.\textsuperscript{47} Abnormal placentation is probably an important etiological factor in women who deliver small infants, as hypoperfusion would restrict fetal growth.\textsuperscript{46} Conversely, inadequate placental development may not contribute to the disease process among women who deliver large babies.\textsuperscript{47} The increased risk of delivering a large for gestational age infant among women with preeclampsia\textsuperscript{47} likely reflects metabolic abnormalities in a subset of preeclamptic women. Women with preeclampsia are also more likely to develop gestational diabetes,\textsuperscript{19} and gestational diabetes increases the risk of fetal macrosomia.\textsuperscript{48} The risk of delivering an infant with a birth weight greater than two standard deviations above the mean is increased among preeclamptic
women who deliver at term, but not among preeclamptic women who deliver preterm. Women who develop preeclampsia after 37 weeks gestation have a greater body mass index than normotensive pregnant controls, or women who develop preeclampsia prior to 34 weeks gestation. Future studies should test the hypothesis that metabolic risk factors that increase the risk of gestational diabetes, including obesity, increase the risk of macrosomia among women who develop preeclampsia at term.

Each of the five causes of preeclampsia may contribute to maternal endothelial dysfunction. Evidence for endothelial dysfunction in preeclamptic patients includes increased production of vasoconstrictors and coagulants, decreased production of vasodilators, and reduced endothelium-dependent flow-mediated vasodilation. This dysfunction is exacerbated by increased vascular sensitivity to vasoconstrictors, including angiotensin II. The autonomic nervous system adaptations that facilitate reduced peripheral vascular resistance in normal pregnancy are absent in preeclampsia, and resting sympathetic hyperactivity in preeclamptic women may contribute to excessive vasoconstriction and elevated blood pressure.

Many symptoms of preeclampsia are attributable to vascular endothelial dysfunction (Table 2.1). Hypertension results from excessive vasoconstriction and failure to reduce peripheral vascular resistance during pregnancy. Convulsions are a consequence of cerebral haemorrhaging, vasospasm and focal ischemia. Proteinuria reflects increased renal endothelial permeability to large proteins. Increased endothelial permeability also causes edema. Peripheral edema leads to weight gain and swelling of the extremities, whereas cerebral edema can cause headaches and blurred vision. Dyspnea results from pulmonary edema. Abdominal pain occurs when fluids and inflammatory substances leak into the liver. In some cases, maternal endothelial
<table>
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<tr>
<th>Maternal Symptom</th>
<th>Effected Organ</th>
<th>Characteristic of Dysfunctional Endothelium</th>
<th>Pathophysiological Consequence</th>
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<tbody>
<tr>
<td>Hypertension</td>
<td>Vasculature</td>
<td>↓ Vasodilatory capacity ↑ Sensitivity to vasoconstrictors</td>
<td>Inadequate reduction in peripheral vascular resistance, excessive vasoconstriction</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Kidney</td>
<td>↑ Endothelial permeability</td>
<td>Allows passage of large proteins into urine</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>Brain</td>
<td>Vasospasm</td>
<td>Focal ischemia</td>
</tr>
<tr>
<td>Headache, Visual disturbances</td>
<td>Brain</td>
<td>↑ Endothelial permeability</td>
<td>Edema and tissue swelling</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Liver</td>
<td>↑ Endothelial permeability</td>
<td>Edema and tissue swelling</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>Lung</td>
<td>↑ Endothelial permeability</td>
<td>Edema and tissue swelling</td>
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dysfunction reduces utero-placental blood flow and persistent ischemia can cause intrauterine
growth restriction.19

2.4 Prevention and Treatment

The primary method of preventing maternal and fetal mortality and morbidity is delivery,
balancing the maternal and fetal risks of continued gestation against the consequences of
premature birth.19 While there are no proven interventions to reduce the incidence of
preeclampsia or to prolong gestation, recent research has focused on antioxidant supplementation
to reduce oxidative stress. In a controlled randomized trial, Chappell et al.37 demonstrated that
supplementation with antioxidant vitamins C and E lowered the incidence of preeclampsia in
women at risk for the disease. A 21% decrease in the ratio of plasminogen activator inhibitor–1
to plasminogen activator inhibitor-2 suggested that supplementation reduced endothelial
dysfunction. The authors postulated that reducing oxidative stress in early pregnancy may
prevent endothelial dysfunction and the resulting late-stage symptoms of preeclampsia.37

Unfortunately, four recent randomized controlled trials involving over 4,000 women
demonstrated that supplementation with 1000mg of Vitamin C and 400 IU of Vitamin E does not
reduce the incidence of preeclampsia (Table 2.2).58-61 Although trophoblastic invasion is
completed and the maternal blood supply to the placenta is established by 12 weeks gestation,28
supplementation commenced between 14 and 20 weeks gestation.58-61 This may have been too
late to prevent abnormalities in placental development that lead to preeclampsia. Women
receiving supplements had an increased incidence of antenatal hospitalization for hypertension,
and antihypertensive medication prescription in one trial.61 Studies report both an increase59 and
Table 2.2: Trials of antioxidant supplementation to prevent preeclampsia

<table>
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<th>Authors</th>
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<th>Gestational Age at Entry &amp; Supplements</th>
<th>Results</th>
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<td>Rumbold, et al.61</td>
<td>Australian women with normal blood pressure at the beginning of the index pregnancy and at trial entry were randomized to supplementation (n=935) or placebo (n=942). Exclusion criteria: multiple gestation, chronic renal failure, known potentially lethal fetal anomaly, thrombophelia, antihypertensive medication, contraindication to Vitamin C or E supplementation</td>
<td>14-22 weeks Vitamin C: 1000mg Vitamin E: 400 IU</td>
<td>Vitamin C &amp; E supplementation had no effect on the incidence of preeclampsia (RR 1.20, 95% CI 0.82-1.75), the risk of death or serious outcomes for the infant (RR 0.79, 95% CI 0.61-1.02) or the risk of SGA (RR 0.87, 95% CI 0.66-1.16) Women taking supplements had an increased risk of antenatal hospital admission for hypertension (RR 1.54, 95% CI 1.00-2.39), and antihypertensive medication prescription (RR 1.67, 95% CI 1.03-2.69)</td>
</tr>
<tr>
<td>Beazley, et al.58</td>
<td>Women with previous preeclampsia, chronic hypertension, DM requiring insulin, or multiple gestation, randomized to supplementation (n=52) or placebo (n=48)</td>
<td>14-20 weeks Vitamin C: 1000mg Vitamin E: 400 IU</td>
<td>Supplementation with Vitamins C and E had no effect on the incidence of preeclampsia (Supplement group: 17.3%, Placebo group: 18.8%)</td>
</tr>
<tr>
<td>Poston, et al.59</td>
<td>Women with ≥1 risk factors (previous HELLP syndrome, preeclampsia requiring delivery at &lt;37 weeks in the pregnancy before the index pregnancy, current or previous medicated essential hypertension, DM requiring insulin, chronic renal disease, abnormal uterine artery Doppler waveform, multiple pregnancy, primiparity and a BMI &gt; 30 kg/m², diastolic blood pressure &gt; 90 mmHg before 20 weeks gestation in current pregnancy) randomized to supplementation (n=1199) or placebo (n=1205)</td>
<td>14-21 weeks Vitamin C: 1000mg Vitamin E: 400 IU</td>
<td>Vitamins C and E supplementation had no effect on the incidence of preeclampsia (RR 0.97, 95% CI 0.80-1.17) Women taking supplements were significantly more likely to deliver a low birthweight baby (RR 1.15, 95% CI 1.02-1.30), however the incidence of small for gestational age babies did not differ between supplementation and placebo groups (RR 1.12, 95% CI 0.96-1.31)</td>
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</table>
Brazilian women diagnosed with preeclampsia in the pregnancy prior to the index pregnancy, or chronic nonproteinuric hypertension, and randomized to supplementation (n=355) or placebo (n=352). Exclusion criteria: multiple gestation, contraindication to Vitamin C or E supplementation, use of aspirin or an anticoagulant, DM, known fatal fetal anomaly, 24 hour proteinuria > 300mg.

Supplementation with Vitamins C and E had no effect on the incidence of preeclampsia (RR 0.87, 95% CI 0.61-1.25), or perinatal outcomes, including the incidence of low birth weight, SGA, fetal or neonatal deaths, neonatal morbidity, preterm delivery, or low APGAR scores at 1 and 5 minutes.

Indonesian women with low antioxidant status ([SOD] < 1102 U/gHb or 164 U/ml) assigned to supplement (n = 29) or placebo (n = 31). Exclusion criteria: history/current use of antihypertensive or diuretic, IVF pregnancy, >150mg Vitamin C or 75 IU Vitamin E supplement/day, placental or fetal abnormality, NSAID use, uterine bleeding within 1 week of screening, history of medical complications, uterine malformation

Significant reduction in rate of preeclampsia (OR 0.18), abortion, placental abruption, preterm delivery, intrauterine fetal death in supplementation group

Blood pressure at delivery was reduced among the supplementation group (systolic 118 ± 11 vs 133 ± 24 for control; diastolic 78 ± 8 vs. 86 ± 13 for control)

Abbreviations: IU, international units; RR, relative risk; CI, confidence interval; SGA, small for gestational age; DM, diabetes mellitus; IVF, in vitro fertilization; NSAIDs, non-steroidal anti-inflammatory drugs; HELLP, hemolysis, elevated liver enzymes, low platelets; SOD, superoxide dismutase.
no change\textsuperscript{60} in the risk of delivering a low birth weight baby among women in the supplement group compared to the placebo group.

In contrast, a small study examining the effects of antioxidant supplementation in 60 Indonesian women with low antioxidant status reported both a decreased incidence of preeclampsia (OR 0.18) and reduced blood pressure at delivery in the supplementation group (Table 2).\textsuperscript{62} Several design factors may have contributed to these differing results. First, supplementation included a wide variety of antioxidant nutrients in addition to vitamins C and E. This may have resulted in a broad-spectrum upregulation of antioxidant systems which was not achieved by supplementation with vitamins C and E alone. Second, supplementation was initiated at 8-12 weeks gestation, compared with 12-22 weeks in the larger studies.\textsuperscript{58-61} Supplementation in the first trimester may prevent adverse effects of oxidative stress during placental formation,\textsuperscript{62} or may facilitate antioxidant upregulation and reduce oxidative stress before placental and/or maternal tissue damage occurs. Third, the study was restricted to women with low antioxidant status at entry.\textsuperscript{62} The pathophysiology of preeclampsia is heterogeneous, and the relative contributions of each of the various underlying causes of preeclampsia in women with different risk factors are not known. The broad range of risk factors used to select candidates for other trials\textsuperscript{58-61} may therefore have resulted in the inclusion of women for whom oxidative stress contributes minimally to the pathophysiological process, while excluding women at risk for preeclampsia due to low antioxidant status. When participants of one large trial were stratified by risk factor at enrollment, however, there were no differences in the incidence of preeclampsia between the supplement and placebo groups.\textsuperscript{59} A large randomized controlled trial is required to determine whether the preventative effects of supplementation with a wide variety of antioxidant nutrients initiated in the first trimester observed in this small study\textsuperscript{62} are due to Type 1 error.
2.5 Prenatal Physical Activity and Preeclampsia Risk: Epidemiology and Randomized Controlled Trials

Retrospective case-control studies indicate that regular leisure time physical activity (LTPA) in early pregnancy is associated with a 25-60% reduction in the risk of preeclampsia (Table 2.3) depending on the amount, intensity and timing of regular LTPA with respect to the index pregnancy. While some studies indicate that women who engage in the highest intensity exercise or have the greatest weekly energy expenditure for LTPA demonstrate the lowest incidence of preeclampsia, others show no change in risk with increasing activity. Daily physical activity such as frequent walking, stair climbing, or lifting in nulliparous women were also associated with a reduced incidence of preeclampsia. However, the results of these retrospective studies may have been biased due to increased participation by healthy active women, or by reduced activity levels in mid-gestation due to early symptoms among subjects who later developed preeclampsia.

Three recent prospective studies have produced conflicting results. The first reported that any activity during the year prior to conception was associated with a 45% reduction in preeclampsia risk (0.55 (odds ratio (OR)); 0.30-1.02 (95% confidence interval)) among 2,241 American women after adjusting for confounding variables. Similar relationships were observed when physical activity was quantified by minutes/week, energy expenditure, and rating of perceived exertion. In contrast, two larger studies observed no protective effect of exercise during early pregnancy. Compared to sedentary controls, participation in any exercise during early pregnancy had no effect on preeclampsia risk (0.94; 0.85-1.04) in 59,573 Norwegian women. Activity (min/week) in early pregnancy also had no effect on the risk of developing all subtypes of preeclampsia in a sample of 85,139 Danish women. Compared with sedentary women, the risk of severe
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<tr>
<td>Osterdal et al.</td>
<td>85,139 Danish women</td>
<td>Prospective Study Phone interview at 12 weeks gestation: type, frequency and duration of each activity in pregnancy used to calculate minutes/wk of activity, MET scores</td>
<td>No effect of activity (min/week) on risk for all PE subtypes Increased risk of severe PE among women exercising 270-419 min/wk (OR 1.65), and ≥420 min/week (OR 1.78); after adjusting for confounding variables. Similar increase in risk of severe PE among primiparous women, and women with a BMI ≤25. Among women who were unexposed to cigarette smoke in pregnancy, physical activity of ≤270 minutes per week was protective (OR 0.43-0.59), whereas the risk of severe PE was increased among women exercising 270-419 min/wk (OR 2.35), and ≥420 min/week (OR 3.48); after adjusting for confounding variables.</td>
</tr>
<tr>
<td>Magnus et al.</td>
<td>Norwegian women 57,258 NPW, 2,315 women with PE (655 with severe PE, 1,356 with mild PE)</td>
<td>Prospective Study Questionnaire completed at 14-22 wks gestation (median 17 wks); Women were asked about the frequency of 11 activities during pregnancy; monthly frequency scores were summed across all activities</td>
<td>After adjusting for smoking, parity, pre-conception BMI, and other confounding variables, the OR for PE among women who exercised ≥25 times/month was 0.79 (95% CI 0.65-0.96) when compared with sedentary women; OR for women who did any exercise was 0.94 when compared to sedentary women Mild PE: OR 0.84 among women exercising ≥25 times/month, general trend towards decreasing OR with increasing activity Severe PE: OR 0.78 among women exercising ≥25 times/month, no trend towards decreasing OR with increasing activity Clear risk reduction with increasing activity among women with BMI ≤25, increased risk with increasing activity among women with BMI ≥30</td>
</tr>
<tr>
<td>Rudra et al.</td>
<td>2,241 American women, of whom 111 developed PE</td>
<td>Prospective Study Questionnaire completed at 15 wks gestation concerning physical activity in the year</td>
<td>Any activity during the year prior to conception was associated with a 45% risk reduction (OR 0.55) after adjusting for confounding variables. Similar relationships were observed when physical activity was quantified by minutes/week, energy expenditure, and rating of perceived exertion.</td>
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prior to pregnancy; subjects reported modality, frequency (sessions/wk, months/year), duration, and rating of perceived exertion; second questionnaire concerning activity during the week before the interview

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Results</th>
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<tbody>
<tr>
<td>Rudra et al. 23</td>
<td>740 NPW, 244 women with PE</td>
<td>Physical activity during one week in early pregnancy not associated with PE risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-significant increase in risk among women who were sedentary before conception, but started exercising in early pregnancy (OR 2.03) compared to women who were inactive at both time periods, however this conclusion was based on 9 cases and 74 controls. This effect was not observed among women who exercised prior to and during the first trimester (OR 0.76) or stopped exercise in the first trimester (OR 0.73).</td>
</tr>
<tr>
<td>Saftlas et al. 24</td>
<td>2,422 NPW, 172 women with GH, 44 women with PE</td>
<td>Women who classified their usual exertion as moderate (OR 0.54), strenuous (OR 0.33), or very strenuous (OR 0.22) were less likely to develop PE than women who did not engage in physical exertion. Similar, but weaker, relationships were observed for energy expenditure. Very strenuous or maximal usual exertion was associated with reduced PE risk among women who did (OR 0.33) and did not (0.28) meet physical activity guidelines.</td>
</tr>
<tr>
<td>Sorensen et al. 25</td>
<td>Cases: 201 preeclamptic women without HELLP Controls: 356 NPW</td>
<td>Any LTPA reduced the risk of PE (OR = 0.66). Any PA in the first 20 wks of pregnancy decreased PE risk by 34%. Light/moderate PA reduced risk by 24%. Vigorous PA reduced risk by 54%. Maximal intensity and number of sessions / wk inversely related to PE risk</td>
</tr>
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</table>
Irwin et al.\textsuperscript{21} 5,605 active duty U.S. Navy personnel, including women with GH (n = 244), mild (n = 182) and severe (n = 33) PE, eclampsia (n = 7), unspecified hypertension (n = 28) Diagnosis and occupation determined from database; occupations classified according to physical activity exposure. Nulliparous women: High levels of occupational lifting significantly reduced PE risk (RR = 0.68). Non-significant trend towards reduced PE risk among women whose jobs required more standing, lifting, exertion and industrial machinery use. Parous women: Moderate occupational exertion and lifting increased PE risk.

Marcoux et al.\textsuperscript{22} Cases: Women with PE (n = 152) and GH (n = 251) Controls: 505 NPW Post-partum interview and questionnaire concerning LTPA in the first 20 weeks of pregnancy The RR of PE decreased as hours of PA / wk increased. Inverse relationship between the RR of PE and maximal LTPA intensity.

Abbreviations: NPW, normotensive pregnant women; GH, gestational hypertension; PE, preeclampsia; LTPA, leisure time physical activity; wks, weeks; PA, physical activity; OR, odds ratio; RR, relative risk; HELLP, hemolysis, elevated liver enzymes, low platelets.
Preeclampsia was increased among women exercising 270-419 min/wk (1.65; 1.11-2.43), and ≥420 min/week (1.78; 1.07-2.95), after adjusting for confounding variables.\(^7\)

While the Danish and Norwegian studies did not observe a protective effect of exercise during early pregnancy in the entire sample, protective effects were observed in specific subpopulations\(^5\) or at specific dosages.\(^6,7\) The risk of preeclampsia was also reduced among Norwegian women who exercised ≥25 times/month during early pregnancy, compared to sedentary controls (All subtypes: 0.79; 0.65-0.96, Severe: 0.78; 0.54, 1.13).\(^6\) In Danish women who were not exposed to smoke during pregnancy, women who exercised less that 270 minutes/week had a reduced risk of severe preeclampsia (adjusted ORs 0.43-0.59, depending on activity), whereas women who exercised more than 270 minutes/week had an increased risk of severe preeclampsia (adjusted ORs 2.35-3.48, depending on activity).\(^7\) Other studies have demonstrated that women who smoke during pregnancy are less likely to develop preeclampsia than women who do not smoke during pregnancy.\(^63\) Additional studies are needed to determine whether these effects are due to Type II error.

Prospective studies suggest that exercise during the year prior to conception protects against preeclampsia risk,\(^5\) however exercise in early pregnancy generally does not.\(^5,7\) The American study found no effect of activity during one week in early pregnancy on preeclampsia risk, but did observe a protective effect of pre-pregnancy exercise.\(^5\) This may support a differential effect of pre-pregnancy vs. early pregnancy exercise, or indicate that exercise during one week in early pregnancy is not representative of exercise during the first trimester. Future studies must conclusively determine whether pre-pregnancy and early pregnancy exercise have different effects, as these results are critically important in identifying physiological mechanisms. The absence of a protective effect of exercise during early pregnancy suggests that preeclampsia risk
is not influenced by the acute effects of exercise, short term training adaptations, or the impact of exercise on pregnancy-specific processes (i.e. early placental development). If exercise during the year prior to conception is associated with a reduced risk of preeclampsia, but exercise during early pregnancy is not, then the protective effect is likely mediated by long-term physiological adaptations associated with increased fitness. Associations between preeclampsia risk and exercise in early pregnancy may therefore be obscured by changes in activity levels following conception. The proportion of inactive women in the American study increased (9% vs. 26%), and the proportion of women exercising >7 hours/week decreased (28% vs. 0%), during early pregnancy compared to pre-pregnancy measurements. Pre-pregnancy and early pregnancy activity will not necessarily be proportionate. Women may decrease, eliminate, or change the modality of exercise due to complications, nausea, or concerns about safety, or start/increase exercise participation to improve health during pregnancy. Activity levels in early pregnancy may therefore be less representative of physical fitness than exercise during the year prior to conception.

Unfortunately, each of these three studies has significant limitations. The Norwegian study only measured exercise frequency, and did not account for intensity or duration. This is analogous to examining the effectiveness of a drug by measuring the frequency with which patients took the drug, without accounting for the dose that each patient received. Without accounting for exercise duration and intensity, it cannot be determined whether the results are valid. The American study included only 2300 women, and did not have sufficient power to examine relationships in mild vs. severe preeclampsia. Furthermore, the assessment of physical activity during early pregnancy only included the week prior to the study, which may not have been representative of activity levels throughout the first trimester. The Danish study consistently refers to the exposure that they measured as physical activity, however the methods section clearly states that women were
asked whether they participated in any exercise during early pregnancy. This discrepancy suggests that study participants may not have reported physical activity that they did not consider to be exercise. In support of this interpretation, the percentage of inactive controls much greater Danish study (63%) than in the American (Pre-pregnancy, 9%; Early pregnancy, 24%) and Norwegian (26%) studies. Women in the American study were asked about participation in sports and recreation, whereas women in the Norwegian study were asked if they participated in any of the activities on a 14-item list, which include “brisk walking” and “other”.

The measurement of exercise, rather than physical activity, in the Danish study has two important implications. First, it suggests that the control group in the Danish study includes non-exercising women who maintained an active lifestyle, as well as sedentary women. If this interpretation is correct, the Danish study measures the effect of exercise in comparison to non-exercising controls, but does not assess the effect of exercise or physical activity in comparison to a sedentary/inactive lifestyle. A sedentary lifestyle increases the risk of many chronic health conditions, including obesity, hypertension, the metabolic syndrome, cardiovascular disease, and Type II diabetes, whereas modest amounts of physical activity (3 hours of brisk walking/week) substantially reduce the risk of most chronic health conditions. Second, it suggests that the 270 minute/week threshold above which the risk of severe preeclampsia increases includes only exercise, and not all forms of physical activity. Furthermore, the 270 minute/week threshold requires verification. This threshold was not derived from analyses treating weekly exercise duration as a continuous variable, but was based on five exercise duration categories designed to include the activity durations most commonly reported by study participants. The categories below and above the 270 minutes/week threshold included all values between 150-269, and 270-419 minutes/week, respectively. These two categories span a 269 minute/week difference in activity levels; therefore it is unlikely that increases in the risk of preeclampsia occurred at 270
minutes/week. Additional studies are therefore needed to verify the increased risk of severe preeclampsia among women participating in high volumes of exercise, and to determine the actual weekly exercise duration at which the risk of severe preeclampsia increases.

The lack of consensus and significant limitations to each of the three prospective studies demonstrate the need for carefully designed studies examining the relationship between preeclampsia and exercise. Large, prospective studies which include pre-pregnancy and early pregnancy activity measurements are needed to determine whether the divergent results of the American\textsuperscript{5} and Danish\textsuperscript{7} studies reflect a true differential effect of early pregnancy vs. pre-pregnancy activity, or were due to limitations of one or both of these studies. Physical activity and exercise participation are very difficult to measure accurately, and measurements of physical fitness (VO\textsubscript{2} max) often provide a clearer assessment of the relationship between chronic activity and disease risk. Future studies should use inactive or sedentary control subjects to avoid confounding by potential beneficial effects of maintaining a physically active lifestyle on the relationship between preeclampsia and exercise. Studies that assist researchers in determining which of the proposed pathophysiological mechanisms contributed to the development of preeclampsia in individual women would facilitate the development of targeted preventive interventions for different subtypes of preeclampsia. Due to the heterogenous nature of the pathophysiology of the syndrome, it is unlikely that a single preventive or treatment strategy will be effective in all women. Existing prospective studies suggest that exercise in early pregnancy does not protect against preeclampsia in the general population.\textsuperscript{5,7} Although available evidence indicates that exercise during the year prior to pregnancy protects against preeclampsia, larger studies in diverse populations are needed to confirm this relationship, and to determine whether it applies to the risk of both mild and severe preeclampsia.\textsuperscript{5}
Large-scale clinical trials examining the relationship between regular exercise and preeclampsia have not yet been conducted. A small clinical trial, however, compared the effects of walking and stretching programs initiated at 18 weeks gestation on the incidence of preeclampsia among inactive women with a previous personal history of the disease. Women randomized to the walking intervention (n = 41) were asked to walk for 40 minutes five days per week, at an intensity defined by 55-69% of maximum heart rate, 50-74% of peak VO2, and a rating of perceived exertion of 12-13 (somewhat hard) on a 6-20 Borg scale. Women in the stretching program (n = 38) were asked to complete a 40 minute video of stretching exercises, designed to maintain heart rate within 10% of resting levels, five times per week. The mean number of sessions completed ranged between 2.5 and 4.5 sessions per week, and decreased in both groups as pregnancy progressed. Exercise duration in the walking group decreased from 36 ± 6 minutes at 18 weeks gestation to 31 ± 12 minutes during the last week of the intervention, and the percentage of exercise performed within the target heart rate range decreased from 35 ± 32% at 18 weeks gestation, to 17 ± 25% during the last week of the intervention. Preeclampsia developed in 14.6% (95% confidence interval 5.6-29.2%) of women in the walking group and 2.6% (95% confidence interval 0.1-13.8%) of women in the stretching group, whereas gestational hypertension was reported in 22.0% (95% confidence interval 8.7-35.2%) of women in the walking group and 39.5 (95% confidence interval 23.2-55.8%) of women in the stretching group. Differences between groups were not significant, as a sample size of 101 subjects per group would have been required to detect a difference in the incidence of preeclampsia with an alpha of 0.5 and 80% power. Research is needed to determine whether an exercise program initiated in mid-pregnancy should have reduced preeclampsia risk, as epidemiological studies have only examined the effects of exercise performed during the year prior to pregnancy, the first trimester, or the first 20 weeks of pregnancy. The pathophysiological placental events that lead to preeclampsia occur during the first trimester, and were likely established before
training adaptations that may have halted or attenuated these pathophysiological processes occurred.

Although not significant, the incidence of preeclampsia among women randomized to the stretching program was lower than would be expected among women who developed preeclampsia in a previous pregnancy.\textsuperscript{65} It is unlikely that these women were more active due to an increased energy expenditure for activities of daily living, as women in the walking group took an average of approximately 2,000 additional steps per day at 18 and 28 weeks gestation, and 1,000 additional steps per day in the last week of the intervention.\textsuperscript{65} The intervention program included only 200 minutes/week of exercise, which is below the 270 minutes per week reported to increase the risk of severe preeclampsia in one study.\textsuperscript{7} Further investigation is required to determine whether these results may have been due to Type 1 error, or demonstrate that high-risk women who begin an exercise program in mid-pregnancy are more likely to develop preeclampsia.

2.6 Postulated Mechanisms for a Protective Effect

Physical conditioning has beneficial effects on many physiological systems that are adversely affected in preeclampsia. Four separate but potentially interactive mechanisms are postulated by which regular exercise prior to conception,\textsuperscript{5} or in early pregnancy among specific subpopulations\textsuperscript{7} or at specific dosages,\textsuperscript{6,7} may protect against preeclampsia (Figure 2.1):\textsuperscript{3,4}

\textit{Enhanced Placental Growth and Vascularity:} Abnormal placental development is an underlying cause of preeclampsia in some women, particularly those with growth-restricted fetuses.
Figure 2.1: Postulated etiology of preeclampsia and proposed benefits of exercise
Solid-line boxes: Effects of preeclampsia; Dashed-line boxes: Effects of Exercise; (?) indicate pathways by which genetics may influence preeclampsia risk. Abbreviations: AT\textsubscript{1}-AA, angiotensin-1 autoantibodies.
Inadequate trophoblastic invasion of the uterine spiral arteries in early pregnancy may lead to an incomplete loss of sensitivity to vasoconstrictors in maternal myometrial vessels, causing intermittent hypoxia and reperfusion. Repeated hypoxia and reperfusion increases pro-oxidant production, and anti-oxidant deficiency may impair the ability of the mother to dispose of large quantities of ROS released following reperfusion. ROS may also enhance release of placental villous tissue fragments, elevating systemic inflammation and endothelial activation. Release of any of these substances (cytokines, lipid peroxides, ROS, elevated concentrations of villous tissue fragments, fetal DNA) into the maternal circulation may cause the systemic oxidative stress that contributes to endothelial dysfunction and preeclampsia.

Exercise during early pregnancy has been reported to decrease the risk of preeclampsia among women exercising more than 25 times/week, and decrease the risk of severe preeclampsia among women who are unexposed to smoke in pregnancy, and exercise less than 270 minutes/week. Beneficial effects of regular exercise on placental growth may contribute to a reduced risk of preeclampsia in these subgroups. Regular exercise in early pregnancy stimulates placental growth in healthy women, and may protect against the pathophysiological placental changes that lead to preeclampsia. Ultrasound measurements show that placental volumes and growth rates in mid-pregnancy are increased among women who begin an exercise program in early pregnancy, compared to non-exercising controls with an active lifestyle. In addition, point-counting techniques performed on randomly sampled sections of fixed placentas demonstrate a reduced fraction of nonfunctional tissue and an increased volume of villous tissue in exercising women at term. The increase in villous tissue volume was primarily attributable to an increased volume of terminal villi, which mediate exchange between maternal and fetal blood. Enhanced placental
growth and vascularity improves perfusion and transport capacity, and this adaptive response to exercise may prevent reductions in fetal substrate and oxygen supplies during intermittent decreases in placental blood flow. Parenchymal volume and total vascular volume are increased in placentas of women who stop exercising after 20 weeks gestation compared to non-exercising controls. Placentas of women who continue regular exercise until term demonstrate further increases in the growth of the intervillous space, and stem and intermediate villi. The absence of differences in terminal villous volume suggests that early pregnancy is a critical period for exercise-induced stimulation of placental growth. Existing studies have been conducted in healthy low-risk women, and future studies should determine whether they also apply to women at risk for poor placental growth.

**Prevention/Reduction of Oxidative Stress:** Regular exercise enhances antioxidant defense systems, limiting cellular damage from exercise-induced oxidative stress. Animal models indicate that exercise training upregulates antioxidants in the liver, heart, and skeletal muscle, while increasing the activities of the antioxidant enzyme superoxide dismutase (SOD) and the non-enzymatic antioxidant glutathione peroxidase. Among healthy men and women, a 16 week aerobic training program increased whole blood glutathione peroxidase activity and plasma glutathione reductase activity without altering erythrocyte SOD activity. The training program also increased LDL resistance to oxidation, resulting in a decrease in oxidized LDL concentration. Elevated energy expenditure for low-intensity physical activity in Spanish women is associated with high erythrocyte SOD activity, while elevated energy expenditure for high-intensity activity is associated with high glutathione peroxidase activity. Although erythrocyte activities of the antioxidant enzymes SOD, glutathione peroxidase, and catalase do not differ between active and sedentary pregnant women prior to delivery, SOD and catalase activities increase dramatically one hour post-partum in active women. This increase is not
observed in sedentary women, and appears to prevent labor-induced increases in malondialdehyde (an indicator of lipid peroxidation). These results suggest that regular exercise training may enhance maternal antioxidant responses to increased oxidative stress in normal pregnancy, which could prevent endothelial dysfunction and the resulting symptoms of preeclampsia.

**Reduction of Inflammation:** Although the effects of physical activity on inflammatory markers during pregnancy are not known, most studies support a systemic anti-inflammatory effect of regular exercise in non-pregnant individuals and patients with heart failure and coronary artery disease. Women in the highest quintile of physical activity demonstrated a 68% reduction in C-reactive protein (CRP) levels in comparison with women in the lowest quintile. Moderate associations between physical activity and both CRP and interleukin-6 were markedly attenuated after adjustment for BMI and leptin, an indicator of fat mass, suggesting that the anti-inflammatory effects of exercise are mediated through changes in body composition. The effects of exercise training on CRP may also depend on baseline CRP levels. A 20 week training program decreased CRP in white and black women with high CRP levels (>3.0 mg/L), however there were no changes in women with baseline CRP < 3.0 mg/L. Although reductions in CRP following 12 weeks of exercise training in men and women with coronary artery disease were greater among diabetic patients than among non-diabetic patients, this difference was likely attributable to a 36% greater mean baseline CRP in diabetic patients. The training program also decreased interleukin-1, interleukin-6, and interferon γ, while increasing the anti-inflammatory cytokine interleukin-10. If exercise training has similar anti-inflammatory effects in pregnant women, this could prevent or attenuate the systemic inflammatory response that occurs in preeclampsia.
Correction of Endothelial Dysfunction: The effects of regular exercise on endothelial function in healthy pregnant women and women at risk for preeclampsia have not been examined. Aerobic conditioning, however, increases local endothelium-dependent dilation in patients with endothelial dysfunction resulting from aging\textsuperscript{81} and type 2 diabetes,\textsuperscript{82} and large muscle mass exercise improves systemic endothelial function in heart failure patients.\textsuperscript{83} Enhanced endothelial function may provide short-term compensation for exercise-induced increases in shear stress.\textsuperscript{84} Researchers have proposed that repeated exposure to shear stress may cause structural vascular remodeling to reduce shear stress, and that endothelial function may return to pre-training levels following vascular remodeling in healthy subjects.\textsuperscript{85} In contrast, exercise training causes long-term improvements in endothelial responses in patients with endothelial dysfunction resulting from diabetes and heart failure.\textsuperscript{82, 83} If similar results are observed in women at risk for preeclampsia, training-induced correction of disease-related endothelial dysfunction may prevent or attenuate the main pathological process leading to symptoms of preeclampsia.

2.7 Effects of Acute Exercise on Oxidative Stress and Circulating Angiogenic Markers
A recent study hypothesized that acute exercise may exacerbate processes that lead to preeclampsia, increasing the risk of severe preeclampsia among women who exercise more than 270 minutes/week in early pregnancy.\textsuperscript{7} The authors focused on increased oxidative stress during and following acute exercise,\textsuperscript{7} however, the effects of acute exercise on other pathophysiological processes associated with preeclampsia should also be examined. Given that sFlt-1 and sEng are believed to explain many of the symptoms of severe preeclampsia, the evidence that release of these substances during acute exercise could increase the risk of severe preeclampsia will also be reviewed.
The effects of acute exercise on oxidative stress have been extensively studied in non-pregnant men and women, and the results of over 300 publications were recently summarized in a comprehensive review article. The conclusions are summarized briefly here, however the reader should refer to the original article for a detailed review and extensive reference list. Acute exercise increases production of reactive oxygen species, which can lead to oxidative stress. Oxidative stress is more likely to occur following longer duration, and/or higher intensity exercise, however the magnitude of the effect also depends on the type of exercise (aerobic vs. anaerobic), and the training status and dietary antioxidant intake of subjects. Lipid peroxidation and protein oxidation increase following acute aerobic exercise, although increases in lipid peroxidation are not observed in all studies and may be intensity dependent. Antioxidant capacity typically decreases during and immediately after aerobic exercise, however increases beyond pre-exercise baseline levels during recovery. Superoxide dismutase, glutathione peroxidase and catalase activities do not change in some studies, however decrease during or immediately after aerobic exercise and rebound during recovery, in others. Transient oxidative stress induced by acute exercise of less than two hours in duration facilitates upregulation of antioxidant defense systems when exercise is repeated regularly. The oxidative stress response to acute aerobic exercise is similar in men and women, however, no study has examined the effects of pregnancy, or pregnancy and physical activity, on the oxidative stress response to acute exercise. Studies in healthy pregnant women, and pregnant women at risk for preeclampsia, are needed to determine whether acute exercise during early pregnancy causes a sustained increase in oxidative stress. Studies should also determine whether upregulation of anti-oxidant enzymes occurs following regular exercise in non-pregnant individuals also occurs during pregnancy.
The effects of acute exercise on circulating angiogenic markers have received limited attention. Acute exercise decreases human skeletal muscle VEGF protein and increases mRNA, however the systemic effects of exercise have not been conclusively determined. Some studies report increased plasma or serum VEGF following acute exercise, while others observed no change, or decreased VEGF and increased sFlt-1. Increases in sFlt-1 following maximal treadmill testing in 5 healthy men were strongly correlated with total peak VO2 consumption ($r^2=0.82$), however, sFlt-1 returned to resting levels within two hours of exercise cessation. The absence of sustained increases in sFlt-1 following short duration, high-intensity exercise are consistent with reports that resting sFlt-1 did not differ between sedentary and exercise trained men (n=8/group). Baseline sFlt-1 tended to increase following two weeks of maximal exercise training in three sedentary men, however differences were not significant due to the number of subjects. Dramatic increases in exercise participation could therefore increase baseline sFlt-1, however, this effect should be confirmed in a larger sample, and may not apply to a moderate intensity exercise program, or to trained subjects.

Although acute exercise and short term exercise training may briefly alter VEGF and sFlt-1 in non-pregnant individuals, available research suggests that circulating levels return to normal following chronic exercise adaptation. Only two of the studies conducted in healthy volunteers included female subjects, and none were pregnant. The effects of acute exercise on placental release of sFlt-1 and sEng are therefore unknown. The application of existing results to pregnancy, a health state characterized by elevated resting sEng, sFlt-1, and decreased free VEGF due to increased VEGF binding activity, should therefore be examined. The generalizability of existing studies is also limited by sample sizes of 5 to 8 subjects per group, and high inter-individual variability. Studies in non-pregnant and pregnant women, and women at risk for preeclampsia, are therefore needed to test the hypothesis.
that anti-angiogenic adaptations resulting from a high exercise volume in early pregnancy may increase the risk of severe preeclampsia.

**2.8 Exercise Safety and the Potential Role of Exercise as an Adjunct Therapy in Pregnancy-Induced Hypertensive Disorders**

Treatment guidelines for gestational hypertension and mild preeclampsia have traditionally recommended bed rest to prevent blood pressure increases associated with daily activity. However, as many as one third of women fail to comply with bed rest recommendations, and compliance does not affect pregnancy outcome among women who develop mild preeclampsia later in gestation. Recent recommendations have therefore shifted towards ambulatory management with careful patient monitoring. The patient is advised to incorporate additional rest into her normal routine, and is carefully monitored to ensure that her condition is stable. Exertion associated with activities of daily living is highly variable. Research is therefore needed to assess the safety of moderate intensity physical activity for both the hypertensive mother and her fetus. Research is also needed to determine whether the blood pressure lowering effects of acute exercise extend to women with gestational hypertension, mild preeclampsia, and chronic hypertension in pregnancy. Although exercise is a recommended adjunct therapy in non-pregnant hypertensive patients, it is not known whether this is a safe or effective adjunct therapy in pregnancy associated hypertensive disorders.

Six studies have conducted brief exercise interventions in women with gestational hypertension and preeclampsia. Comparison of sodium clearance rates in preeclamptic and normotensive pregnant women at rest and during 10-16 minutes of supine cycling suggested that exercise-induced increases in leg blood flow are accompanied by small decreases in uterine blood flow. The authors speculated that the magnitude of the observed reductions would not adversely effect
the fetus unless the mother was severely preeclamptic.\textsuperscript{110} Preeclampsia, however, was defined exclusively by new onset hypertension with no criteria for multisystem organ involvement.\textsuperscript{110}

A second study demonstrated that uteroplacental resistance increases during supine cycling in women with normal pregnancies, pregnancy-induced hypertension, and small for gestational age fetuses.\textsuperscript{109} The authors postulated that this could be detrimental to the fetus, particularly in mothers with an abnormal resting uteroplacental waveform.\textsuperscript{109} However, fetal responses were not directly measured and uteroplacental resistance rapidly returned to baseline levels following exercise.\textsuperscript{109} Differences between complicated and uncomplicated pregnancies may have reflected between-group differences in physical fitness and a failure to standardize the exercise protocol, as cycling tests were completed at the same absolute work rate and exercise duration was not reported.\textsuperscript{109}

Only one study evaluated fetal responses following exercise in hypertensive pregnant patients. Examination of fetal heart rate tracings after five minutes of cycle ergometry in 44 women with high-risk pregnancies (diabetes (n = 9), intrauterine growth restriction (IUGR) (n = 18), hypertension (n = 17)) revealed nine cases of fetal bradycardia.\textsuperscript{112} Unfortunately, the diagnoses of the women whose fetuses developed bradycardia were not reported, and it is unclear whether hypertensive and diabetic women were also screened for IUGR.\textsuperscript{112} Post-exercise bradycardia may be more likely in growth restricted fetuses,\textsuperscript{115} as the chronic placental blood flow deficiency can be exacerbated by diversion of blood from the uterus to the exercising muscles. A subsequent publication evaluating changes in umbilical artery blood flow after five minutes of cycle ergometry in 17 in-patients with hypertension and 2+ proteinuria concluded that five minutes of moderate intensity cycle-ergometry was not harmful to the hypertensive mother or her fetus.\textsuperscript{113} Changes in heart rate, blood pressure, stroke volume, cardiac output, and vascular resistance
during three minutes of isometric handgrip exercise did not differ between healthy pregnant controls and women with gestational hypertension\textsuperscript{111} and mild preeclampsia.\textsuperscript{114}

Existing research has several limitations that should be addressed. First, available studies\textsuperscript{109-114} have not adequately distinguished between gestational hypertension, chronic hypertension and preeclampsia. The etiology of these disorders may differ, and the effects of exercise in each condition should be examined separately. Second, fetal monitoring is needed to directly assess fetal exercise safety in each of these conditions. Third, the potential benefits of exercise as an adjunct anti-hypertensive therapy have not been examined. The maximum exercise duration was 10-16 minutes\textsuperscript{110} and the five-minute exercise bouts used in most studies\textsuperscript{111, 113, 114} are substantially less than the 30 minute minimum recommended to reduce blood pressure in non-pregnant hypertensive patients.\textsuperscript{108} Guidelines for non-pregnant patients also recommend large muscle mass exercise,\textsuperscript{108} although isometric handgrip exercise consistently lowers blood pressure in hypertensive patients.\textsuperscript{116} Observations that diastolic blood pressure in pregnant hypertensive patients fell below pre-exercise levels after supine cycling\textsuperscript{110} should be evaluated by ambulatory blood pressure monitoring. The effects of longer duration, large muscle mass exercise on maternal blood pressure, fetal heart rate and pregnancy outcome have important implications for the management of women with gestational hypertension, chronic hypertension and mild preeclampsia. Study design and selection of patients must be conducted carefully, however, as exercise is contraindicated for women who have intrauterine growth restriction, severe hypertension or preeclampsia, or in hypertensive patients whose condition is not stable.\textsuperscript{117} Exercise protocols should be designed in accordance with the Canadian Guidelines for Exercise During Pregnancy and the Post-partum Period (Table 2.4).\textsuperscript{117, 118}
Table 2.4: Canadian guidelines for aerobic exercise during pregnancy

**Screening for Contraindications**

Women with absolute contraindications should not exercise. Absolute contraindications are ruptured membranes or premature labour, persistent 2nd or 3rd trimester bleeding/placenta previa, pregnancy-induced hypertension or preeclampsia, incompetent cervix, evidence of intrauterine growth restriction, high order pregnancy (triplets or more), and uncontrolled Type 1 diabetes, hypertension or thyroid disease, other serious cardiovascular, respiratory or systemic disorder.

Women with relative contraindications should talk to their health care provider to determine whether the benefits of exercise outweigh the risks. Relative contraindications include a history of spontaneous abortion or premature labour in previous pregnancies, mild/moderate cardiovascular or respiratory disease (ie. chronic hypertension or asthma), anemia or iron deficiency (Hb < 100 g/L), malnutrition or eating disorder, twin pregnancy after 28th week, or another significant medical condition.

**Aerobic Exercise Guidelines**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>3 – 4 sessions/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity</td>
<td>RPE of 12-14 on the 6-20 BORG scale, corresponding to a verbal descriptor of somewhat hard</td>
</tr>
<tr>
<td>Target heart rate zones for pregnant women: Age &lt;20, 140-155 bpm; Ages 20-29, 135-150 bpm; Ages 30-39, 130-145 bpm</td>
<td></td>
</tr>
<tr>
<td>Use the talk test to avoid over-exertion</td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Weight-supported or low impact large muscle mass exercise. Pregnant women should avoid contact sports, SCUBA diving, exercise in warm, humid environments, and activities that require fast direction changes or present a danger of falling</td>
</tr>
<tr>
<td>Time</td>
<td>≥15 minutes per session; research demonstrates that sessions of up to 30 minutes are safe</td>
</tr>
<tr>
<td>Additional Precautions</td>
<td>Pregnant women should stop exercise and consult a health care provider if they experience excessive shortness of breath, chest pain, painful uterine contractions (&gt;6-8/hour), vaginal bleeding, “gush” of fluid from vagina, dizziness or faintness</td>
</tr>
</tbody>
</table>

Guidelines for muscular conditioning are described in the ParMED-X for Pregnancy. Abbreviations: Hb, hemoglobin; RPE, rating of perceived exertion; bpm, beats per minute.
2.9 Conclusions

The etiology of preeclampsia has not been clearly established, but likely involves an interaction between impaired placental development, maternal constitutional factors, genetic susceptibility, inflammation, oxidative stress, and endothelial dysfunction. Although there is no proven method of preventing preeclampsia, retrospective case-control,\textsuperscript{23, 25} and prospective epidemiological\textsuperscript{5} studies indicate that regular exercise during the year prior to conception may protect against preeclampsia. Participation in any regular exercise during early pregnancy does not alter preeclampsia risk in general,\textsuperscript{5-7} but may reduce preeclampsia risk in specific subpopulations\textsuperscript{5} or at specific dosages.\textsuperscript{6-7} One study reported an increased risk of severe preeclampsia among women who exercised more than 270 minutes/week in early pregnancy, compared to sedentary controls.\textsuperscript{7}

Future research should examine three key issues. First, the differential effect of exercise performed during the year prior to conception, vs. exercise in early pregnancy, must be evaluated in large studies which assess physical activity during both time periods. Second, studies should test the hypothesis that regular exercise prior to pregnancy, or during early pregnancy in specific subpopulations or at specific dosages, may prevent preeclampsia by enhancing placental vascular growth, reducing inflammation, increasing the concentration and activity of antioxidants to reduce oxidative stress, and correcting endothelial dysfunction. Third, the hypothesis that the acute effects of exercise may increase the risk of severe preeclampsia by exacerbating pathophysiological processes associated with preeclampsia should be examined.
2.10 References


Chapter 3
Regular Exercise Reduces sFlt-1 and Increases PlGF in Late Gestation

3.1 Abstract
Preeclampsia affects 2-7% of pregnancies, is a leading cause of maternal and fetal morbidity and mortality, and may be caused by anti-angiogenic processes, including excessive increases in soluble endoglin (sEng) and soluble fms-like tyrosine kinase-1 (sFlt-1). Compared to sedentary controls, prospective studies observed protective effects of regular\textsuperscript{1} and frequent\textsuperscript{2} exercise against preeclampsia, and a reduced risk of severe preeclampsia among women who were not exposed to smoke and exercised <270 minutes/week.\textsuperscript{3} The objective of this study was to examine the effects of acute and chronic exercise on angiogenic markers. sEng, sFlt-1, and placental growth factor (PlGF) were measured before and after moderate-intensity exercise in non-smoking pregnant (16 active, 9 inactive, 34.1±1.6 weeks gestation), and non-pregnant (15 active, 12 inactive) women. Serum sFlt-1 and sFlt-1:PlGF were elevated, and serum PlGF was reduced, in inactive pregnant women compared to active women. Three inactive pregnant participants had high sEng levels compared to the remaining pregnant subjects. Compared to inactive pregnant women, sFlt-1:PlGF and PlGF were not different in women who exercised ≥270 minutes/week, but were significantly decreased in women who exercised <270 minutes/week. Acute, moderate-intensity exercise in the third trimester did not cause anti-angiogenic changes.

Keywords: angiogenesis, exercise, pregnancy, preeclampsia
3.2 Introduction

Preeclampsia affects 2-7% of pregnancies,\textsuperscript{4,5} and is a leading cause of maternal and fetal morbidity and mortality.\textsuperscript{6} The only cure is urgent delivery, often at the risk of iatrogenic prematurity. Early retrospective case-control studies indicated that women who exercised regularly were 25-60% less likely to develop preeclampsia, depending on the amount, intensity and timing of physical activity and exercise with respect to pregnancy.\textsuperscript{7-10} Three recent prospective studies, however, have produced conflicting results.\textsuperscript{1-3} The first reported that any activity during the year prior to conception was associated with a 45% reduction in preeclampsia risk (0.55 (OR); 0.30-1.02 (95% confidence interval)) among 2,241 American women after adjusting for confounding variables.\textsuperscript{1} Similar relationships were observed when physical activity was quantified by minutes/week, energy expenditure, and rating of perceived exertion.\textsuperscript{1} In contrast, two larger studies observed no protective effect of exercise during early pregnancy.\textsuperscript{2,3} Compared to sedentary controls, participation in any exercise during early pregnancy had no effect on preeclampsia risk (0.94; 0.85-1.04) in 59,573 Norwegian women.\textsuperscript{2} Activity (min/week) in early pregnancy also had no effect on the risk of developing all subtypes of preeclampsia in a sample of 85,139 Danish women.\textsuperscript{3} Compared with sedentary women, the risk of severe preeclampsia was increased among women exercising 270-419 min/wk (1.65; 1.11-2.43), and \geq 420 min/week (1.78; 1.07-2.95), after adjusting for confounding variables.\textsuperscript{3}

While the Danish and Norwegian studies did not observe a protective effect of exercise during early pregnancy in the entire sample, protective effects were observed in at specific dosages or among specific subpopulations.\textsuperscript{2,3} In Danish women who were not exposed to smoke during pregnancy, women who exercised less than 270 minutes/week had a reduced risk of severe preeclampsia (0.43-0.59, depending on activity), whereas women who exercised more than 270 minutes/week had an increased risk of severe preeclampsia (OR 2.35-3.48, depending on
activity). The risk of preeclampsia was also reduced among Norwegian women who exercised ≥25 times/month during early pregnancy, compared to sedentary controls (All subtypes: 0.79; 0.65-0.96, Severe: 0.78; 0.54, 1.13). Several factors may have contributed to these divergent results, including differences in sample size, study populations, timing of the physical activity measurement (pre-conception vs. early pregnancy), method of physical activity quantification, subtype of preeclampsia (mild vs. severe), and selection of confounding variables. Additional large, prospective studies are needed to clarify the relationship between preeclampsia and exercise, however, physiological studies are also needed to examine the effects of regular exercise on the pathophysiological processes that lead to preeclampsia.

Although the etiology of preeclampsia is incompletely defined, recent evidence suggests that excessive circulating soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) contribute to the pathophysiology of the syndrome. sFlt-1 is the only known receptor for placental growth factor (PIGF) and one of three receptors for vascular endothelial growth factor (VEGF). sFlt-1 reduces angiogenesis by binding these ligands as a non-signaling decoy, and causes hypertension, proteinuria, and glomerular endotheliosis in rats. Serum sFlt-1 is elevated five-fold in women with preeclampsia compared with normotensive pregnant women. Decreases in unbound serum VEGF and PIGF in preeclamptic women are proportional to elevations in sFlt-1. These antiangiogenic effects of sFlt-1 may impair pregnancy-induced adaptation in maternal myometrial vessels, and contribute to systemic maternal endothelial dysfunction. sEng is the extra-cellular domain of Endoglin (Eng), a co-receptor for transforming growth factor (TGF) β1 and β3. sEng inhibits binding of TGF β1 to Eng, preventing eNOS activation and subsequent vasodilation. Combined sEng and sFlt-1 overexpression in rats causes symptoms of severe preeclampsia, including proteinuria, severe hypertension, intrauterine growth restriction, and low
platelet counts. Serum sEng concentrations are elevated three-fold in women with mild preeclampsia, and five-fold in women with severe preeclampsia, compared to normotensive pregnant women. Despite the prominence of sFlt-1, PI GF and sEng in preeclampsia research, no study has compared levels of angiogenic markers between active and sedentary non-pregnant, pregnant, or preeclamptic women. This information is essential to determine whether any effect of regular exercise on preeclampsia risk may be mediated through angiogenic markers. Furthermore, researchers have hypothesized that acute exercise may exacerbate the pathophysiological processes that lead to preeclampsia, explaining the increased in the risk of severe preeclampsia among women exercising more than 270 minutes/week reported in one study. Research examining both the acute and chronic effects of exercise on angiogenic markers during pregnancy is therefore needed.

This study is the first to examine the effects of acute and chronic exercise on sFlt-1, PI GF and sEng in healthy, age-matched, non-smoking pregnant and non-pregnant women. The objectives were to determine whether serum sFlt-1, sFlt-1:PI GF and sEng differ between active and sedentary women in late gestation, and to assess the effects of acute exercise on serum sEng, sFlt-1, VEGF, and PI GF in non-smoking pregnant and non-pregnant women. It was hypothesized that serum VEGF, PI GF, sFlt-1, sFlt-1:PI GF and sEng do not differ between active and inactive pregnant women at 30-36 weeks gestation, and that these markers do not change immediately following acute moderate intensity exercise in late gestation.

3.3 Methods
Subjects were 16 active pregnant (AP), 9 inactive pregnant (IP), 15 active non-pregnant (ANP), and 12 inactive non-pregnant (INP) healthy, non-smoking women, age 22 to 40 years, who were
not taking hormonal contraception or medications. Women with cardiovascular disease or hypertension, previous pregnancy-induced hypertension, or symptoms of hypertension or proteinuria during the current pregnancy were excluded. Inactive women did not regularly exercise at an intensity that was sufficient to cause sweating, while active women exercised for at least three hours/week. Pregnant women were between 30 and 36 weeks gestation. Non-pregnant women were tested between menstrual cycle days 20 and 28. Testing was completed as part of a larger study examining the effects of pregnancy, acute exercise, and chronic activity on endothelial function.

**Pre-test Screening:** Women completed standardized screening forms (Physical Activity Readiness Questionnaire or PARmed-X for Pregnancy, http://www.csep.ca/forms.asp) to confirm that they had no contraindications to exercise. The doctor or midwife of each pregnant participant reviewed the form to verify that she could exercise safely. A transabdominal ultrasound was performed to ensure that the pregnancy was singleton and uncomplicated, and that the fetus was not small for gestational age. An obstetrician (G.A.L. Davies) provided medical clearance for exercise testing after examining the screening form and ultrasound results. The Health Sciences Research Ethics Board at Queen’s University approved the study protocol, and all subjects provided written, informed consent prior to participating.

**Physical Activity Indices:** Subjects completed a 3-day physical activity record on consecutive days (one weekend day, two weekdays) within 2 weeks of the test to evaluate current physical activity. Daily energy expenditure (DEE) and maximum voluntary physical activity (MVPA) were calculated as described previously.
Chronic physical activity was evaluated using the Kaiser Physical Activity Survey (KPAS), which was validated in non-pregnant\textsuperscript{15} and pregnant\textsuperscript{16} women. The KPAS was delivered once to non-pregnant subjects, to assess activity during the past year, and twice to pregnant subjects, to assess activity during pregnancy and for one year before conception. Physical activity indices for Household & Family Care, Occupational, Active Living, Sports & Exercise, and Total Activity were calculated as described previously.\textsuperscript{15, 16} Weighted total activity during pregnancy was computed by weighting each index by its average contribution to the energy expenditure of pregnant women (Household & Family Care: 50\%, Occupational: 20\%, Active Living: 25\%, Sports & Exercise: 5\%).\textsuperscript{16}

**Exercise Test:** Subjects avoided caffeine and strenuous exercise for 12 and 24 hours prior to testing, respectively, and consumed a standardized meal at the test site at 9:45am. Immediately after the meal, an obstetric nurse drew a resting blood sample from a vein in the subject’s forearm. The participant sat quietly for 10 minutes while heart rate (Polar Vantage Heart Rate Monitor) and breath-by-breath respiratory measurements (VMax II, Cardinal Health or Moxus Modular Metabolic System, AEI Technologies) were collected. After a 3-minute warm-up on the cycle ergometer (Sensor Medics Model 800S, Cardinal Health), women completed a 90-second ramp work rate increase until she reached a rating of perceived exertion (RPE) of 13 on the 6-20 Borg scale. This intensity was maintained for 20 minutes. RPE was the primary indicator of intensity as RPE during weight-supported exercise is not effected by pregnancy.\textsuperscript{17} Intensity was confirmed by a steady-state heart rate of approximately 130 beats/minute in non-pregnant subjects, and 140 beats/minute in pregnant subjects, to account for the pregnancy-induced 10-15 beat/minute increase in resting heart rate.\textsuperscript{17} A post-exercise blood sample was collected by
venipuncture within three minutes of exercise cessation. Pregnancy outcome data were obtained from medical records.

**Blood Biochemistry:** Blood samples for serum VEGF, PlGF, sFlt-1 and sEng determination were collected in serum separator tubes (Becton Dickson VACUTAINER Systems), clotted on ice for 1.5-2 hours, and centrifuged at 4°C for 10 min at 2500 rpm. Serum was collected, frozen at -80°C, and subsequently assayed. Serum concentrations of unbound VEGF, unbound PlGF, and sFlt-1 were determined in triplicate, and serum concentrations of sEng were measured in duplicate by ELISA using commercially available kits (R&D Systems). Kit interassay coefficients of variation were 7% for VEGF, sFlt-1 and sEng, and 9% for PlGF.

**Statistical Analysis:** The distribution of all variables was examined prior to analyses, and serum VEGF, PlGF, sFlt-1 and sEng were log-transformed to minimize the effects of positive skewing on statistical tests. The effects of chronic activity on gestational age, menstrual cycle day, weighted total activity, gestational age at delivery, and birth weight were determined using independent-samples t-tests. The effects of pregnancy and activity on the odds of delivering a male infant, and parity, were examined using chi-squared tests. Comparisons of MVPA in active and inactive subgroups were performed using Wilcoxin’s Rank-Sum test. The effects of pregnancy and chronic activity on all remaining subject characteristics and physical activity indices were assessed using a 2 x 2 analysis of variance (ANOVA) with pregnancy (non-pregnant vs. pregnant) and activity (active vs. inactive) as between-subjects factors. Pregnancy-induced changes in BMI and chronic physical activity indices were examined using a 2 x 2 repeated measures ANOVA, with pregnancy (pre-conception vs. pregnant) as the within-subjects factor, and activity as the between-subjects factor. The effects of acute exercise, pregnancy, and chronic
physical activity on angiogenic markers were assessed using a 2 x 2 x 2 repeated-measures analysis of variance, with acute exercise as the within-subjects factor (pre vs. post), and pregnancy and activity as the between-subjects factors. Where significant main effects were present, simple main effects were determined using paired or independent samples t-tests with the Sidak correction for multiple comparisons.

Pearson correlations were used to examine the strength of the relationships between physical activity indices and pre-exercise VEGF, PI GF, sFlt-1, sFlt-1:PIGF and sEng. Separate analyses were performed for pregnant and non-pregnant women using pooled data from active and inactive groups. High Cook’s distance and Centered Leverage values were used to identify outliers, which were removed from individual analyses. Partial correlations were computed to control for BMI in non-pregnant women, and BMI, fetal weight, and gestational age in pregnant women. Partial correlations between PI GF and physical activity indices also controlled for sFlt-1. All analyses were performed using SPSS 16.0 (SPSS Inc.). Statistical significance was determined by a two-sided p value < 0.05.

3.4 Results

Subject Characteristics and Pregnancy Outcome: Age did not differ between groups (Table 3.1). Gestational age and cycle day were similar in pregnant and non-pregnant groups, respectively. Compared to active controls of the same reproductive status, BMI was significantly greater in inactive women. BMI during pregnancy increased significantly from pre-conception values in both active and inactive pregnant women. Inactive women were more likely to be multiparous, however the magnitude of this effect was similar in pregnant and non-pregnant subgroups.
Table 3.1: Physical Characteristics of Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active Pregnant (n = 16)</th>
<th>Inactive Pregnant (n = 9)</th>
<th>Active Non-pregnant (n = 15)</th>
<th>Inactive Non-pregnant (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.0 ± 3.7</td>
<td>31.2 ± 3.9</td>
<td>32.8 ± 5.1</td>
<td>33.1 ± 4.4</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>34.0 ± 1.3</td>
<td>34.4 ± 2.1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Cycle Day</td>
<td>N/A</td>
<td>N/A</td>
<td>25 ± 2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-conception / Non-pregnant</td>
<td>22.7 ± 2.9</td>
<td>25.7 ± 4.6†</td>
<td>22.6 ± 2.8</td>
<td>26.1 ± 4.0†</td>
</tr>
<tr>
<td>Late Gestation</td>
<td>27.3 ± 2.7*</td>
<td>31.2 ± 5.9*‡</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Parity (Percent nulliparous)</td>
<td>81%</td>
<td>56%‡</td>
<td>87%</td>
<td>33%‡</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Abbreviations: BMI, body mass index.
Significant difference from preconception: *p<0.01.
Significant difference from active group of same reproductive status: †p<0.05.

Low mean voluntary physical activity and Sports & Exercise Index scores confirm that inactive pregnant women did not exercise regularly during pregnancy, and inactive non-pregnant women did not exercise regularly during the year prior to the study (Table 3.2). In contrast, the high scores obtained in active women confirm that active pregnant women exercised regularly throughout pregnancy and during the year prior to conception, whereas active non-pregnant women exercised regularly during the year prior to the study. Compared to inactive women, significant increases in mean voluntary physical activity and the Sports & Exercise Index demonstrate that exercise participation was greater in active women. The Active Living Index was also significantly increased among active non-pregnant women, and active pregnant women during pregnancy, compared to inactive controls. This indicates that active women spent more time engaged in active transportation, and less time watching television, than inactive women.
Table 3.2: Physical activity characteristics of subjects

<table>
<thead>
<tr>
<th>Current Activity</th>
<th>AP</th>
<th>IP</th>
<th>ANP</th>
<th>INP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Energy Expenditure (kcal)</td>
<td>3236 ± 579</td>
<td>3295 ± 573</td>
<td>2955 ± 585</td>
<td>3265 ± 915</td>
</tr>
<tr>
<td>Daily Energy Expenditure (kcal/kg/15 minutes)</td>
<td>42 ± 3*</td>
<td>39 ± 2†</td>
<td>47 ± 5</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>Mean Voluntary Physical Activity (kcal)</td>
<td>257 ± 131</td>
<td>7 ± 22‡</td>
<td>578 ± 314</td>
<td>61 ± 148‡</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chronic Activity Indices</th>
<th>AP</th>
<th>IP</th>
<th>ANP</th>
<th>INP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household and Family Care</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>2.5 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>2.3 ± 0.4</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Pregnant</td>
<td>2.3 ± 0.4‡</td>
<td>2.3 ± 0.3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Occupational</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>2.7 ± 0.8</td>
<td>2.7 ± 0.7</td>
<td>2.5 ± 0.6</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>Pregnant</td>
<td>2.5 ± 0.8</td>
<td>2.9 ± 0.8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Active Living</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>3.4 ± 0.7</td>
<td>3.1 ± 0.5</td>
<td>3.8 ± 0.9</td>
<td>2.7 ± 0.6‡</td>
</tr>
<tr>
<td>Pregnant</td>
<td>3.0 ± 0.4†</td>
<td>2.4 ± 0.6‡</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Sports and Exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>4.4 ± 0.4</td>
<td>2.8 ± 1.3‡</td>
<td>4.4 ± 0.4</td>
<td>1.6 ± 0.6‡</td>
</tr>
<tr>
<td>Pregnant</td>
<td>4.1 ± 0.3</td>
<td>1.6 ± 0.8†</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Total Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>13.0 ± 1.1</td>
<td>11.1 ± 1.5‡</td>
<td>12.9 ± 1.3</td>
<td>9.2 ± 1.6‡</td>
</tr>
<tr>
<td>Weighted Total Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>10.4 ± 1.2</td>
<td>9.7 ± 0.9</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Current activity data were available for 9 out of 12 INP subjects, whereas Chronic Activity Indices were available for 11 out of 12 INP subjects. Abbreviations: N/A, not applicable.

Significant difference from non-pregnant group of same activity level: *p<0.01, †p<0.05.
Significant difference from active control group of same reproductive status: ‡p<0.01, §p<0.05.
Significant difference from preconception: ¶p<0.01, ‖p<0.05.
Pregnancy outcome data were unavailable for one active woman who delivered at home. One inactive woman developed gestational hypertension. All participants delivered healthy infants after 37 weeks gestation. Birth weight (Active: 3584g (Mean) ± 355g (SD); Inactive: 3540g ± 292g), gestational age at delivery (Active: 39.9 ± 1.2; Inactive: 39.5 ± 1.3), and the odds of carrying a male fetus (Active: 40%, Inactive: 44%) did not differ between active and inactive women.

**Effects of Acute Exercise on Heart Rate and Respiration:** Acute exercise significantly increased heart rate, $\dot{V}O_2$/kg, $\dot{V}O_2$, $\dot{V}CO_2$, ventilation ($\dot{V}_E$), tidal volume and breathing frequency in all subject groups (p<0.001 for all main effects and pairwise comparisons, Table 3.3). Two observations indicated that physical fitness was greater among active pregnant and non-pregnant women compared to inactive women. First, significantly lower resting heart rate among active pregnant (p=0.021) and non-pregnant (p<0.001) women, compared to inactive women, are consistent with training bradycardia. Second, although all four groups exercised at the same relative intensity, as measured by RPE, absolute exercise intensity was greater in active pregnant and non-pregnant women than in inactive women. Work rate was significantly increased in active groups compared to inactive groups. $\dot{V}O_2$ and $\dot{V}CO_2$ did not differ significantly between active and inactive women at rest, but were significantly increased among active groups during exercise due to larger exercise-induced increases in $\dot{V}O_2$ and $\dot{V}CO_2$ in active women.

**Effects of Acute Exercise, Chronic Inactivity and Pregnancy on Levels of Angiogenic Markers:** In the inactive pregnant participant who later developed gestational hypertension,
<table>
<thead>
<tr>
<th></th>
<th>AP (n = 16)</th>
<th>IP (n = 9)</th>
<th>ANP (n = 15)</th>
<th>INP (n = 12)</th>
<th>AP (n = 16)</th>
<th>IP (n = 9)</th>
<th>ANP (n = 15)</th>
<th>INP (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Workload (Watts)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>78 ± 13</td>
<td>51 ± 11§</td>
<td>94 ± 28</td>
<td>60 ± 21§</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>85 ± 10†</td>
<td>93 ± 11§,†</td>
<td>62 ± 8</td>
<td>77 ± 6§</td>
<td>142 ± 13*§,†</td>
<td>143 ± 15*</td>
<td>130 ± 14*</td>
<td>137 ± 18*</td>
</tr>
<tr>
<td>$\dot{V}O_2$/kg</td>
<td>3.9 ± 0.5</td>
<td>3.5 ± 0.4</td>
<td>4.1 ± 0.6</td>
<td>3.7 ± 0.4</td>
<td>17.6 ± 2.4*§,†</td>
<td>12.6 ± 2.3*§,†,§,ǁ</td>
<td>22.2 ± 4.5*</td>
<td>16.4 ± 2.4*§,§</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L/min)</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.26 ± 0.04</td>
<td>0.26 ± 0.04</td>
<td>1.36 ± 0.19*</td>
<td>1.05 ± 0.11*§,§,ǁ</td>
<td>1.45 ± 0.31*</td>
<td>1.12 ± 0.29*§,§</td>
</tr>
<tr>
<td>$\dot{V}CO_2$ (L/min)</td>
<td>0.27 ± 0.03</td>
<td>0.28 ± 0.04</td>
<td>0.23 ± 0.04</td>
<td>0.24 ± 0.04</td>
<td>1.34 ± 0.19*</td>
<td>1.09 ± 0.12*</td>
<td>1.46 ± 0.33*</td>
<td>1.14 ± 0.29*§,§</td>
</tr>
<tr>
<td>$V_E$ (L/min)</td>
<td>10.7 ± 1.0†</td>
<td>11.7 ± 2.4†</td>
<td>8.8 ± 1.6</td>
<td>9.1 ± 1.3</td>
<td>44.1 ± 8.6*</td>
<td>39.2 ± 6.6*</td>
<td>39.8 ± 8.1*</td>
<td>34.0 ± 8.5*</td>
</tr>
<tr>
<td>Tidal volume (L)</td>
<td>0.72 ± 0.16</td>
<td>0.72 ± 0.10</td>
<td>0.60 ± 0.13</td>
<td>0.67 ± 0.18</td>
<td>1.61 ± 0.37*</td>
<td>1.42 ± 0.39*</td>
<td>1.39 ± 0.24*</td>
<td>1.33 ± 0.43*</td>
</tr>
<tr>
<td>Breathing frequency (breaths/min)</td>
<td>16 ± 3</td>
<td>17 ± 5</td>
<td>15 ± 3</td>
<td>14 ± 3</td>
<td>28 ± 5*</td>
<td>30 ± 9*</td>
<td>29 ± 4*</td>
<td>26 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Abbreviations: RPE, rating of perceived exertion; N/A, not applicable; AP, active pregnant; IP, inactive pregnant; ANP, active non-pregnant; INP, inactive non-pregnant.

Significant difference from rest: *p<0.001.

Significant difference from non-pregnant group of same activity level: †p<0.01, ‡p<0.05.

Significant difference from active group of same reproductive status: §p<0.01, †p<0.05.
sFlt-1 levels were similar to the maximum values observed in the active and inactive pregnant groups, PlGF levels were similar to the minimum values observed in the active and inactive pregnant groups, and sEng levels were within the normal range for the inactive pregnant group. Data from this woman were therefore included in all analyses.

Unbound VEGF was not detectable in sera from any pregnant woman in late gestation (Table 3.4). Similar results have been reported previously\textsuperscript{18} due to increased VEGF binding activity resulting from increased sFlt-1.\textsuperscript{11, 12} In non-pregnant women, VEGF decreased immediately post-exercise in active (p=0.008) and inactive (p=0.010) non-pregnant women. There was no main effect of activity (p=0.200) on VEGF, and no interaction between exercise and activity (p=0.200).

Compared to non-pregnant controls, concentrations of PlGF, sFlt-1 and sEng were significantly greater in pregnant women pre and post-exercise (p<0.001 for main effects and pairwise comparisons, Table 3.4). The concentration of PlGF was significantly lower in inactive pregnant women pre (p=0.014) and post-exercise (p=0.009) compared to active pregnant women (Main effect of activity, p=0.036; pregnancy x activity, p=0.081). Although PlGF levels tended to increase post-exercise (Main effect, p=0.031), this effect only reached statistical significance in active pregnant women (p=0.014). PlGF was significantly correlated with sFlt-1 (r=-0.550, p=0.005), whereas partial correlations controlling for BMI, birth weight, and gestational age did not reach statistical significance (r=-0.426, p=0.061).

Compared to active women, sFlt-1 levels were greater in inactive women (Main effect of activity p=0.010), however pairwise comparisons only reached statistical significance among pregnant women pre (p=0.005) and post-exercise (p=0.017). Exercise significantly increased sFlt-1 levels
Table 3.4: Effects of pregnancy, regular exercise, and acute exercise on markers of angiogenesis

<table>
<thead>
<tr>
<th>Marker</th>
<th>Time Point</th>
<th>AP</th>
<th>IP</th>
<th>ANP</th>
<th>INP</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/ml)</td>
<td>Rest</td>
<td>ND</td>
<td>ND</td>
<td>107 ×/÷ 2.3</td>
<td>158 ×/÷ 1.9</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>ND</td>
<td>ND</td>
<td>57 ×/÷ 2.3</td>
<td>80 ×/÷ 2.6</td>
</tr>
<tr>
<td>PI GF (pg/ml)</td>
<td>Rest</td>
<td>366 ×/÷ 2.2*</td>
<td>209 ×/÷ 1.5*</td>
<td>12.9 ×/÷ 1.20</td>
<td>12.3 ×/÷ 1.27</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>414 ×/÷ 2.2*</td>
<td>227 ×/÷ 1.6*</td>
<td>13.3 ×/÷ 1.17</td>
<td>12.5 ×/÷ 1.24</td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>Rest</td>
<td>3181 ×/÷ 1.9*</td>
<td>5313 ×/÷ 1.2*</td>
<td>85 ×/÷ 1.2</td>
<td>92 ×/÷ 1.1</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>3467 ×/÷ 1.8*</td>
<td>5356 ×/÷ 1.3*</td>
<td>90 ×/÷ 1.3</td>
<td>107 ×/÷ 1.2</td>
</tr>
<tr>
<td>sFlt-1:PI GF</td>
<td>Rest</td>
<td>8.4 ×/÷ 3.6</td>
<td>21.7 ×/÷ 2.2*</td>
<td>6.9 ×/÷ 1.31</td>
<td>7.5 ×/÷ 1.23</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>7.7 ×/÷ 3.5</td>
<td>21.6 ×/÷ 1.9*</td>
<td>7.4 ×/÷ 1.46</td>
<td>8.6 ×/÷ 1.30</td>
</tr>
<tr>
<td>sEng (ng/ml)</td>
<td>Rest</td>
<td>8.09 ×/÷ 1.33*</td>
<td>11.22 ×/÷ 1.83*</td>
<td>4.02 ×/÷ 1.26</td>
<td>4.24 ×/÷ 1.26</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>7.94 ×/÷ 1.32*</td>
<td>11.78 ×/÷ 1.71*</td>
<td>4.08 ×/÷ 1.27</td>
<td>4.43 ×/÷ 1.26</td>
</tr>
</tbody>
</table>

Variables were log-transformed for analysis, and values presented are the back-transformed mean ×/÷ the back-transformed SD, presented as a factor. Abbreviations: ND, not detectable.

Significant difference from non-pregnant group of same activity level: *p<0.01, †p<0.05.
Significant difference from active group of same reproductive status: ‡p<0.01, §p<0.05.
Significant difference from Rest: ǁp<0.01, ¶p<0.05.
in inactive non-pregnant (p=0.012), but not in active non-pregnant (p=0.303), active pregnant (p=0.109), or inactive pregnant (p=0.985) women (Main effect of acute exercise, p=0.020).

sEng concentrations were significantly increased in pregnant women pre and post-exercise compared to non-pregnant controls of the same activity level (p<0.001 for main effect and all pairwise comparisons). sEng concentrations were also significantly greater among inactive pregnant women pre (p=0.025) and post-exercise (p=0.005) compared to active women, however, differences between active and inactive non-pregnant women did not reach statistical significance (Main effect of activity, p=0.021). Acute exercise had no effect on sEng levels (Main effect, p=0.426).

sFlt-1:PlGF tended to be greater in pregnant women than in non-pregnant controls of the same activity level (Main effect of pregnancy, p=0.025), however these differences only reached statistical significance in inactive pregnant women pre (p=0.007) and post-exercise (p=0.018). sFlt-1:PlGF increased significantly following acute exercise in inactive non-pregnant women (p=0.035), but not in the remaining subject groups (Main effect of acute exercise, p=0.415; acute exercise x pregnancy, p=0.037). sFlt-1:PlGF did not differ between active and inactive non-pregnant women, but was significantly increased in inactive pregnant women pre (p=0.012) and post-exercise (p=0.007) compared to active pregnant women (Main effect of activity, p=0.024; activity x pregnancy, p=0.072).

**Effect of Weekly Exercise Duration on Levels of Angiogenic Markers:** Comparisons between inactive pregnant women, active pregnant subjects who exercised for <270 minutes/week (n = 6), and active pregnant subjects who exercised for ≥270 minutes/week (n = 10) revealed significant
differences in PlGF (Main effect, p=0.019, Table 3.5) and sFlt-1:PlGF (Main effect, p=0.019).

Compared to active subjects exercising less than 270 minutes/week, PlGF was significantly lower (p=0.019), and sFlt-1:PlGF was significantly higher (p=0.021) in inactive pregnant women. Decreases in PlGF (p=0.086) and increases in sFlt-1:PlGF (p=0.070) among women exercising ≥270 minutes per week were not statistically significant, compared to women exercising less than 270 minutes/week. The assumption of homogeneity of variance was not met for sEng and sFlt-1. sEng was not statistically different between groups (p=0.220), however, there was a non-significant trend towards an effect of group on sFlt-1 (p=0.067).

Table 3.5: Effects of weekly exercise duration on markers of angiogenesis during pregnancy

<table>
<thead>
<tr>
<th>Marker</th>
<th>Inactive Pregnant (n = 9)</th>
<th>Active Pregnant, &lt;270 min/week (n = 6)</th>
<th>Active Pregnant, ≥270 min/week (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlGF (pg/ml)</td>
<td>209 ×/÷ 1.5*</td>
<td>590 ×/÷ 2.2</td>
<td>275 ×/÷ 1.9†</td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>5313 ×/÷ 1.2</td>
<td>2531 ×/÷ 2.0</td>
<td>3901 ×/÷ 1.7</td>
</tr>
<tr>
<td>sFlt-1:PlGF</td>
<td>21.7 ×/÷ 2.2*</td>
<td>4.0 ×/÷ 3.4</td>
<td>14.4 ×/÷ 2.8†</td>
</tr>
<tr>
<td>sEng (ng/ml)</td>
<td>11.22 ×/÷ 1.83</td>
<td>7.19 ×/÷ 1.26</td>
<td>8.69 ×/÷ 1.35</td>
</tr>
</tbody>
</table>

Variables were log-transformed for analysis, and values presented are the back-transformed mean ×/÷ the back-transformed SD, presented as a factor. Abbreviations: ND, not detectable.

Significant difference from Active pregnant, <270 min/week: *p<0.05.

Non-significant difference from Active pregnant, <270 min/week: †p=0.070, ‡p=0.086.

**Correlations Between Physical Activity and Levels of Angiogenic Markers:** Among non-pregnant women, VEGF, PlGF and sEng were not significantly correlated with markers of acute or chronic physical activity (data not shown). Correlation coefficients and p values for partial correlations controlling for BMI were also not significant. sFlt-1 was negatively correlated with
the Active Living Index (Pearson r: -0.471, p=0.017; Partial correlation controlling for BMI: r-
-0.443, p=0.030). Correlations and partial correlations between sFlt-1 and the remaining physical
activity indicators were not significant.

Among pregnant women, PLGF was significantly correlated with the Household & Family Care
Index (r=0.419, p=0.037) and weighted total physical activity (r=0.484, p=0.014). Partial
correlations controlling for BMI, birth weight, gestational age, and sFlt-1 did not reach statistical
significance (Table 3.6). Partial correlations, controlling for BMI, birth weight, and gestational
age, between sFlt-1, and the Active Living Index (r=-0.377, p=0.069) and Weighted Total
Activity (r=-0.453, p=0.052) did not achieve the selected criteria for statistical significance.
Partial correlations between sFlt-1 and the Sports & Exercise Index (r=-0.364, p=0.125), and
mean voluntary physical activity (r=-0.376, p=0.102) were not significant. The Active Living
Index includes positively-weighted questions quantifying the amount of time spent on active
transportation (walking, biking), and a negatively-weighted question quantifying time spent
watching television. Subdivision of this index into the Active Transportation (average of the three
positively-weighted questions) and TV Viewing components revealed that both contributed to the
correlation between sFlt-1 and the Active Living Index (Active Transportation: r=-0.360,
p=0.119; TV Viewing: r=0.377, p=0.122). The consistency of the strength of correlations across
physical activity indices suggests that remaining physically active throughout pregnancy is
associated with reduced sFlt-1.

Partial correlations between sEng and the Active Living Index were statistically significant (r=-
0.544, p=0.011), whereas partial correlations between sEng and mean voluntary physical activity
Table 3.6: Partial correlations between angiogenic markers and activity in pregnancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Variables</th>
<th>DEE (kcal)</th>
<th>MVPA (kcal)</th>
<th>Household &amp; Family Care</th>
<th>Active Living</th>
<th>Active Transport</th>
<th>TV Viewing</th>
<th>Sports &amp; Exercise</th>
<th>Weighted Total Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI GF</td>
<td>BMI, FW, GA, sFlt-1</td>
<td>-0.25</td>
<td>-0.01</td>
<td>0.40 †</td>
<td>0.18</td>
<td>0.14</td>
<td>-0.14</td>
<td>0.11</td>
<td>0.37</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>BMI, FW, GA</td>
<td>-0.10</td>
<td>-0.38</td>
<td>-0.31</td>
<td>-0.43 †</td>
<td>-0.36</td>
<td>0.38</td>
<td>-0.36</td>
<td>-0.45 †</td>
</tr>
<tr>
<td>sEng</td>
<td>BMI, FW, GA</td>
<td>0.06</td>
<td>-0.37 ‡</td>
<td>-0.03</td>
<td>-0.54 *</td>
<td>-0.40 †</td>
<td>0.55 *</td>
<td>-0.22</td>
<td>-0.38 †</td>
</tr>
</tbody>
</table>

Values presented are the coefficients for partial correlations controlling for the effect of the control variables. 90% confidence limits for all correlation coefficients were approximately ±0.30. The Active Living Index was subdivided into two components. The first (Active Transport) is the average of three positively weighted questions concerning the amount of time spent on active transportation, whereas the second (TV Viewing) represents one negatively weighted question concerning time spent watching television. Abbreviations: BMI, body mass index; FW, fetal weight; GA, gestational age.

Significant partial correlation: *p<0.05.
Non-significant partial correlation: ‡0.05<p<0.01.
(r=-0.370, p=0.099) and Weighted Total Activity (r=-0.382, p=0.096) did not reach statistical significance. The partial correlation between sEng and TV Viewing was statistically significant (r=0.550, p=0.010), whereas the partial correlation between sEng and Active Transportation approached statistical significance (r=-0.395, p=0.076).

**Subdivision of Participants by Active Living and Weighted Total Activity:** The inclusion criterion for exercise divided subjects according to their Sports & Exercise Index scores (Table 3.2), however, the Sports & Exercise Index was not significantly correlated with PlGF or sFlt-1. In contrast, PlGF was significantly correlated with Weighted Total Activity, and partial correlations between sFlt-1 and each of the Active Living Index and Weighted Total Activity approached statistical significance (Table 3.5). Active and inactive pregnant women were therefore pooled, and subdivided according to the median Active Living Index, and Weighted Total Activity, scores. Compared to women with Active Living Index scores greater than or equal to the median value of 2.75, PlGF was significantly decreased, and sFlt-1 and sFlt-1:PlGF were significantly increased, among women who scored below the median Active Living Index value (Figure 3.1). Similarly, women with scores below the median Weighted Total Activity value of 10.24 demonstrated significant decreases in PlGF, and significant increases in sFlt-1 and sFlt-1:PLGF, compared to women who scored above the median value. These differences were greater than those observed between the original active and inactive pregnant groups (Figure 3.1), suggesting that maintaining a physically activity lifestyle attenuates pregnancy-induced increases in sFlt-1.

Pre-exercise sEng did not differ significantly between groups when subjects were subdivided by exercise participation, or by the median Weighted Total Activity or Active Living Index scores.
Figure 3.1: Effect of chronic exercise, weighted total activity, and active living on baseline angiogenic markers in pregnancy

Subjects were divided according to exercise participation (original active vs. inactive groups), median Weighted Total Activity score, and the median Active Living Index score. Gray bars represent the less active group. Outliers indicated on graphs were not included in pairwise comparisons. Significant difference between means: *p<0.01, †p<0.05.
This result contrasts with the significant differences in pre-exercise sEng between active and inactive pregnant women observed in the full model (acute exercise x pregnancy x activity). This discrepancy was likely due to reduced power for the pairwise comparison due to the increased variance in the inactive subgroup. sEng among six inactive pregnant women (Range: 5.6 ng/ml-9.7 ng/ml) was similar to levels reported in active pregnant women (Range: 5.5 ng/ml-12.2 ng/ml). In contrast, elevated levels of sEng were observed among the inactive woman who developed gestational hypertension (16.7 ng/ml) and two additional inactive subjects (25.3 ng/ml, 31.3 ng/ml). Physical characteristics and activity indices did not differ between these subjects and the remaining inactive pregnant subjects. These results suggest that while sEng is not systematically elevated among inactive women, inactive women may be more likely to experience high sEng levels than active women.

3.5 Discussion
This is the first study to examine the effects of acute and chronic exercise on sFlt-1, PlGF and sEng in healthy, age-matched, non-smoking pregnant and non-pregnant women. The results reveal three important physiological findings. First, serum PlGF is reduced, and sFlt-1 and sFlt-1:PlGF are elevated, in sedentary non-smoking pregnant women, compared to exercising women. Decreased unbound PlGF among less active pregnant participants may reflect increased binding due to elevated sFlt-1, as PlGF was negatively correlated with sFlt-1 in pregnancy. Sedentary women may also be more likely to experience elevated sEng in pregnancy, as high sEng levels were observed in three inactive pregnant subjects. Second, the effects of exercise on PlGF and sFlt-1:PlGF differed depending on the amount of weekly exercise. Compared to inactive pregnant women, decreases in sFlt-1:PlGF and increases in PlGF were only significant among women who exercised 180-270 minutes/week throughout pregnancy. sFlt-1:PlGF and PlGF did not differ
significantly between inactive pregnant women, and women who exercised more than 270 minutes/week throughout pregnancy. Third, anti-angiogenic changes that could contribute to preeclampsia were not observed following acute, moderate-intensity exercise in the third trimester. With the exception of significant increases in PlGF among active pregnant women, sFlt-1, PIGF, sFlt-1:PIGF and sEng did not change following acute moderate-intensity exercise.

**Chronic effects of physical activity and exercise on circulating angiogenic makers:** The results of this study indicate that the effects of regular exercise on angiogenic markers could contribute to the reduced risk of preeclampsia among active women,¹ and the decreased risk of severe preeclampsia among women who are unexposed to smoke in pregnancy and who exercise less than 270 minutes per week.³ In addition to the significant differences in circulating angiogenic markers between active and inactive groups, sFlt-1 and sEng were positively correlated with time spent watching television, and negatively correlated with indices of physical activity and exercise. Cross-sectional comparisons between pregnant and non-pregnant women suggest that among active women, proportionate pregnancy-induced increases in sFlt-1 and PIGF result in moderate increases in sFlt-1:PIGF. In contrast, pregnancy-induced increases in sFlt-1 greatly exceed increases in PIGF among inactive women, and the increase in sFlt-1:PIGF is extremely large. Longitudinal studies with additional subjects are required to confirm these results, which suggest that increasing physical activity and exercise and decreasing time spent watching television may attenuate pregnancy-induced increases in sFlt-1, and prevent excessive increases in sEng. Regular physical activity may also attenuate pregnancy-induced increases in sFlt-1. While sFlt-1 and sFlt-1:PIGF were significantly greater in sedentary women than in exercising women, the magnitude of differences between groups increased when women were subdivided by Active Living Index or Weighted Total Activity scores. Future studies should evaluate the independent effects of time spent on sedentary activities, physical activity, and
exercise, on circulating levels of sFlt-1 and sEng, and preeclampsia risk. Many correlations and partial correlations in the present study did not reach statistical significance, and the relationships observed in the present study should therefore be interpreted as preliminary data based on a small sample of women. Larger studies including women with a broad range of exercise levels are needed to increase statistical power.

The clinical significance of the observed effects should be evaluated in larger studies which measure angiogenic markers in normotensive active and inactive pregnant women, and women with preeclampsia. The elevated sEng levels observed in three inactive women in the present study approach previously reported values from women with mild preeclampsia, but are much lower than values reported in women with severe preeclampsia and HELLP syndrome.\textsuperscript{13} sFlt-1 in active and inactive pregnant women was much lower than previously reported values in mild and severe preeclampsia, and HELLP syndrome.\textsuperscript{13} These observations suggest that the variations in angiogenic markers associated with physical activity are not sufficient to cause preeclampsia, but may contribute to preeclampsia in women with other risk factors. The present study included healthy, non-smoking women with no history of hypertension, however, further study is required to determine whether similar results would be observed in women with risk factors for preeclampsia, or in smokers.

**Effects of weekly activity duration on circulating angiogenic markers:** One prospective epidemiological study reported that among women who were unexposed to cigarette smoke during pregnancy, women who exercised less than 270 minutes/week had a reduced risk of severe preeclampsia, whereas women who exercised more than 270 minutes/week had an increased risk of severe preeclampsia, compared to sedentary controls.\textsuperscript{3} The chronic effects of exercise on PI GF and sFlt-1:PI GF in the present study were most pronounced among active
pregnant women exercising less than 270 minutes per week throughout pregnancy, as differences between inactive pregnant women, and pregnant women exercising more than 270 minutes/week, were not significant. These effects could contribute to the reported decrease in the risk of severe preeclampsia among women who were not exposed to smoke, and exercised less than 270 minutes/week during early pregnancy. These results do not explain the dramatic increase in risk among women exercising more than 270 minutes per week, compared to sedentary women, as sFlt-1:PlGF and PI GF did not differ between these two groups. These preliminary results are based on small sample sizes (n=6-10/group), and should be confirmed in larger studies. Furthermore, additional studies are needed to determine the actual weekly exercise duration at which the risk of severe preeclampsia increases. The study which observed an increase in the risk of severe preeclampsia among women exercising more than 270 minutes/week in early pregnancy constructed five exercise duration categories designed to include the activity durations most commonly reported by study participants. The categories below and above the 270 minutes/week threshold included all values between 150-269, and 270-419 minutes/week, respectively. These two categories span a 269 minute/week difference in activity levels; therefore it is unlikely that increases in the risk of preeclampsia occurred at exactly 270 minutes/week.

**Acute effects of exercise on circulating markers of angiogenesis:** Researchers have hypothesized that acute exercise may exacerbate the pathophysiological processes that lead to preeclampsia, contributing to the increased risk of severe preeclampsia among women exercising more than 270 minutes/week. sFlt-1 and sEng are central to the pathophysiology of preeclampsia, and the acute effects of exercise on these markers in pregnant and non-pregnant women has never been examined. The present study demonstrates that anti-angiogenic changes that could contribute to preeclampsia did not occur immediately following 20 minutes of acute, moderate-intensity exercise in the third trimester. With the exception of a significant increase in
PIGF among active pregnant women, sFlt-1, PIGF, sFlt-1:PIGF and sEng did not change significantly following exercise in active or inactive pregnant women. There are three important limitations to this finding. First, angiogenic markers were only measured immediately post-exercise, and it is possible that changes could occur later during the recovery period. sFlt-1 increased significantly 30 minutes following maximal treadmill testing in 5 healthy men, however, responses immediately post-exercise were not measured. Second, longer duration exercise may have had different effects. Although the 20 minutes of exercise participants completed during the present study is within the exercise guidelines for pregnant women, many active women in the present study exceeded this duration during their regular exercise sessions. The acute effects of longer-duration exercise should therefore be examined. Third, higher intensity exercise may have had different effects. Increases in sFlt-1 30 minutes after maximal treadmill testing in 5 healthy men were strongly correlated with total peak VO2 consumption ($r^2=0.82$). sFlt-1 returned to resting levels within two hours of maximal treadmill testing, which is consistent with reports that resting sFlt-1 did not differ between sedentary and exercise trained men (n=8/group). Baseline sFlt-1 tended to increase following two weeks of maximal exercise training in three sedentary men, however, differences were not significant due to the number of subjects. Dramatic increases in exercise participation could therefore increase baseline sFlt-1, however, this effect should be confirmed in a larger sample, and may not apply to a moderate intensity exercise program, to trained subjects, or to pregnant women, whom experience elevated sFlt-1 levels due to increased placental production.

The exercise intensity in the present study was selected on the basis of Canadian guidelines for exercise in pregnancy, which recommend a target RPE of 12-14. Seven out of 16 active pregnant women regularly exceeded this intensity, reporting an RPE of 15-17 for one or more exercise activities. Angiogenic responses to acute, high-intensity exercise should therefore be
examined. The effects of acute exercise on angiogenic markers could also be different in early pregnancy, although PlGF, sFlt-1, and sEng did not change following acute exercise in active non-pregnant women. Future studies should also assess the effects of moderate and high-intensity exercise during pregnancy on other pathophysiological processes that lead to preeclampsia.

Among non-pregnant women, post-exercise increases in sFlt-1 were correlated with VEGF \((r=0.503, p=0.009)\), and may have contributed to post-exercise decreases in serum VEGF. Decreased VEGF and increased sFlt-1 were observed following maximal exercise in men,\(^{19}\) however other studies report no change,\(^ {22,23}\) or increased\(^ {21,24-26}\) circulating VEGF post-exercise. All studies used the same assay; therefore other factors must explain the diverse results. VEGF uptake by non-exercising tissues may decrease circulating VEGF during large muscle mass exercise when samples are not drawn from the vein draining the exercising muscle, as three hours of kicking exercise increased femoral vein plasma VEGF without changing femoral artery plasma VEGF.\(^ {24}\) Post-exercise measurement time may influence results, as the time course of changes in plasma VEGF following submaximal cycling is highly variable.\(^ {21}\) The use of plasma vs. serum also affects VEGF levels. Although plasma and serum VEGF levels are correlated,\(^ {21}\) plasma VEGF is unaffected by the amount of time between collection and centrifugation, whereas serum VEGF increases over two hours of clotting due to platelet VEGF release.\(^ {27}\) Sex could affect exercise-induced changes in serum VEGF, as increases in platelet count following maximal exercise are greater in men than in women.\(^ {22}\) Future studies should systematically assess the effects of each of these factors on exercise-induced changes in circulating VEGF.

This study is the first to demonstrate that serum sFlt-1 and sFlt-1:PlGF are increased, and PlGF is decreased, in inactive pregnant women in late gestation compared to active non-smoking women. Cross-sectional comparisons suggest that among active women, pregnancy-induced increases in
sFlt-1 are proportionate to increases in PI GF, whereas pregnancy-induced increases in sFlt-1 greatly exceed increases in PI GF among inactive women. Sedentary women may also be more likely to experience elevated sEng in pregnancy. The effects of regular exercise on PI GF and sFlt-1:PI GF are more pronounced among active women who exercise for less than 270 minutes/week. Larger studies which measure angiogenic markers in normotensive active and inactive pregnant women, and women with preeclampsia, are required to evaluate the clinical significance of the observed effects. Epidemiological studies are also needed to clarify the relationship between exercise and preeclampsia risk, and to evaluate the independent effects of time spent on sedentary activities, physical activity, and exercise. Anti-angiogenic changes that could contribute to preeclampsia were not observed following 20 minutes of acute, moderate-intensity exercise in the third trimester. Future studies in pregnant women should assess the acute effects of exercise on other pathophysiological processes that lead to preeclampsia.
3.6 References


Chapter 4
True Peak Brachial Artery Flow-Mediated Dilation is not Affected by Pregnancy or Regular Exercise

4.1 Abstract
Previous studies report that brachial artery flow-mediated dilation (FMD) is increased\textsuperscript{1,2} or unchanged\textsuperscript{3-7} at 28-35 weeks gestation compared to non-pregnant controls. Existing studies, however, did not test non-pregnant controls in the mid-late luteal phase, examine the effect of pregnancy on the dilatory shear stimulus, account for physical activity, or control for inter-individual variation in the time to peak dilation\textsuperscript{8} by using continuous post-release diameter measurements to identify true peak FMD.\textsuperscript{1-7} True peak brachial artery FMD was measured in 17 active and 8 sedentary pregnant women (34.1±1.6 weeks gestation), and in 19 active and 11 sedentary non-pregnant women (mid-late luteal phase). Decreased vascular tone secondary to increased shear stress contributes minimally to pregnancy-induced increases in baseline brachial artery diameter, as shear stress removal during distal cuff inflation in pregnant women did not reduce diameter to baseline levels observed in non-pregnant controls. Neither the shear stimulus, nor percent FMD, were affected by pregnancy or regular exercise. Continuous diameter measurements are required to control for delayed peak dilation during pregnancy (57±15, vs. 46±15 seconds, p=0.012), as post-release diameter measured at 60 or 55-65 seconds post-release underestimated FMD to a greater extent in non-pregnant than in pregnant women.

Keywords: Endothelium-dependent flow-mediated dilation, pregnancy, shear rate, brachial artery, physical activity
4.2 Introduction
Cardiovascular adaptations at 36 weeks gestation include a 20% increase in blood volume, 30% increase in cardiac output, 40% decrease in peripheral vascular resistance, and a 10% decrease in mean arterial pressure, compared to preconception measurements. These hemodynamic changes could alter the mechanical (shear stress, transmural pressure) environment experienced by the vascular endothelium, which, in combination with hormonal alterations, might be expected to affect conduit artery structure and function. In support of this, previous studies have observed that brachial artery diameter increases during pregnancy, and have proposed that this is due to shear mediated vasodilation. Furthermore, some studies have reported increases in flow-mediated dilation (FMD) compared to non-pregnant controls. Two studies reported significant increases in flow-mediated dilation (FMD) at 32 and 35 weeks gestation compared to non-pregnant controls. However, five additional studies, three of which were based on the same data set, observed no significant change in FMD at 28 to 36 weeks gestation. Two important methodological factors may have contributed to this lack of consensus. First, existing studies measured post-release diameter at pre-defined time points (i.e. 45 or 60 seconds post-release), rather than using continuous measurements to identify true peak diameter. This approach does not control for the high inter-individual variability in the timing of peak dilation. If pregnancy systematically alters the timing of peak dilation, then differences between groups may be an artifact of the post-release measurement time.

The second factor which likely affected conclusions of previous studies is the failure to control for menstrual cycle phase in non-pregnant controls. Two repeated measures studies reported significant variation in FMD during the menstrual cycle in healthy young women. The first observed increases in FMD during the late follicular (18.2 ± 0.8%) and luteal (17.5 ± 0.7%)
phases compared to the early follicular (11.2 ± 0.6%) phase. The second reported significant decreases in FMD during the early luteal phase (4.2 ± 0.6%) compared to the early follicular (8.8 ± 0.6%), late follicular (10.0 ± 0.7%), and late luteal (8.6 ± 0.9%) phases. These studies suggest that the inclusion of women in the early follicular, or early luteal phases could have decreased percent FMD among non-pregnant controls, increasing the magnitude of the difference between pregnant and non-pregnant women. Most pregnant women experience cyclic variation in FMD during menstrual cycles before conception. Comparisons with non-pregnant subjects in the phase most similar to pregnancy are therefore required to demonstrate that differences in FMD are attributable to pregnancy, and not to menstrual cycle phase. Non-pregnant women were tested in the mid-late luteal phase in this study, as this phase is characterized by the highest progesterone concentrations during the menstrual cycle, and estrogen concentrations are also elevated compared to the early follicular phase. Furthermore, non-pregnant women experience cardiovascular changes during the late luteal phase that are similar to those observed in pregnancy (i.e. decreases in mean arterial pressure and systemic vascular resistance, and increases in cardiac output), but are smaller in magnitude.

Two further limitations of previous research also need to be addressed. First, previous studies did not account for potential, pregnancy-induced changes in the shear stimulus which causes FMD. The shear stimulus which causes FMD is the shear stimulus from the time of cuff release to the time of peak diameter (relevant shear stimulus). The percent increase in peak blood flow from baseline levels can decrease with advancing gestational age, however, no study has measured the shear stimulus. Shear stimulus magnitude needs to be accounted for, as potential pregnancy-induced changes in FMD may reflect differences in dilatory stimulus strength. Second, existing studies have not accounted for physical fitness. Brachial artery diameter is increased in endurance
trained women\textsuperscript{14} and men\textsuperscript{15} compared to sedentary controls. Studies reported either increases\textsuperscript{16} or no change\textsuperscript{15,17} in FMD among active young men compared to inactive controls. The only study conducted in young women observed no effect of regular exercise on FMD in the early follicular phase, however FMD was measured distal to the cuff\textsuperscript{14}. FMD measured distal to the cuff includes dilation caused by ischemia during cuff inflation, as well as dilation in response to increased shear stress following cuff release.\textsuperscript{13}

Based on the above, the objectives were to determine whether pregnancy-induced increases in the baseline shear stimulus contribute to increases in brachial artery diameter, to assess the effects of pregnancy on true peak FMD, and to evaluate the effects of physical activity on baseline brachial artery diameter and FMD. It was hypothesized that pregnancy-induced increases in the baseline shear stimulus do not contribute to increases in brachial artery diameter, and that pregnancy and activity do not effect true peak FMD after accounting for the shear stimulus.

### 4.3 Methods

Brachial artery FMD in response to reactive hyperemia was measured in 17 active pregnant (AP), 8 inactive pregnant (IP), 19 active non-pregnant (ANP), and 11 inactive non-pregnant (INP) women as part of a larger study examining the effects of pregnancy, acute exercise, and chronic exercise on endothelial function. Subjects were recruited through flyers and contact with local obstetricians and midwives. All subjects were healthy, non-smokers between the ages of 23 and 40 who were not regularly taking medications, and had no history of hypertension. Pregnant subjects were tested at 34.1 ± 1.6 (mean ± standard deviation) weeks gestation. Non-pregnant subjects were not taking hormonal contraception, and were tested between days 20 and 28 of their menstrual cycle (mid-late luteal phase). Active women had exercised for three or more hours/week for at least eight months, while inactive women did not regularly exercise at a
sufficient intensity to break into a sweat. The study was approved by the Health Sciences Research Ethics Board at Queen’s University, and all subjects provided written informed consent prior to participating.

**Pre-test Screening:** Non-pregnant women completed a medical screening form (Physical Activity Readiness Questionnaire, http://www.csep.ca/forms.asp) to ensure that they were healthy with no contraindications to exercise. Pregnant women completed a similar standardized form (PARmed-X for Pregnancy, http://www.csep.ca/forms.asp) with their obstetrician or midwife to obtain medical clearance. An abdominal ultrasound was performed to ensure that the subject was having a normal, singleton pregnancy and the fetus was not small for gestational age. An obstetrician (G.A.L. Davies) provided final approval for testing in pregnancy after examining each screening form and ultrasound result.

**Physical Activity Indices:** Subjects completed a 3-day physical activity record\textsuperscript{18} on consecutive days (one weekend day, two weekdays) within 2 weeks of the test to evaluate current physical activity. Daily energy expenditure (DEE) and maximum voluntary physical activity (MVPA) were calculated as described previously.\textsuperscript{18} Chronic physical activity was evaluated using the Kaiser Physical Activity Survey (KPAS), which was validated in non-pregnant\textsuperscript{19} and pregnant\textsuperscript{20} women. The KPAS was delivered once to non-pregnant subjects, to assess activity during the past year, and twice to pregnant subjects, to assess activity during pregnancy and for one year before conception. Physical activity indices for Household & Family Care, Occupational, Active Living, Sports & Exercise, and Total Activity were calculated as described previously.\textsuperscript{19,20} Weighted total activity during pregnancy was
computed by weighting each index by its average contribution to the energy expenditure of pregnant women (Household & Family Care: 50%, Occupational: 20%, Active Living: 25%, Sports & Exercise: 5%).

**Reactive Hyperemia Protocol:** Subjects avoided caffeine and exercise for 12 and 24 hours before the test, respectively, and consumed a standard meal (350 kcal) at 7am. After arriving at the laboratory at 8am, subjects were seated in a semi-darkened room for 20 minutes of quiet rest. The left arm was then positioned on a table at the level of the heart. A blood pressure cuff attached to an automatic inflation device was placed on the forearm distal to the elbow. Beat-to-beat finger arterial pressure was measured continuously using a Finapres (Model 2300, Ohmeda) photoplethysmographic cuff placed around the right middle finger. Continuous, grayscale images of the brachial artery were obtained using a 10 MHz probe operating in B-Mode (GE Vingmed System 5, GE Medical Systems). Simultaneous blood flow velocity measurements were recorded using Doppler ultrasound, with the same probe operating at 4 MHz. An insonation angle of 68° was maintained throughout each trial, to allow for accurate velocity measurements while optimizing image quality by ensuring that the ultrasound beam was perpendicular to the vessel. The probe was positioned over the brachial artery a few centimeters proximal to the cuff, wherever the clearest image was obtained. A one-minute scan was performed to determine resting brachial artery diameter, after which the occlusion cuff was inflated to 250 mmHg for 5 minutes. Brachial artery diameter and blood flow velocity measurements were collected during the last minute before cuff release, and for two minutes following release. Ultrasound images of brachial artery diameter and velocity were recorded on video tape, then transferred to a Digital Imaging and Communications in Medicine (DICOM) file at a rate of 25 frames per second for offline analysis.
Hematocrit (Hct) was determined in a subset of 5 active pregnant, and 5 active and one inactive non-pregnant participants. After the reactive hyperemia protocol, a blood sample from a vein in the forearm was collected in a tube coated with EDTA. Hct was measured at the Kingston General Hospital Core Laboratory. Viscosity was then calculated using the formula for high shear rates (208 s\(^{-1}\)), \[\text{Viscosity} = 0.12 \times \text{Hct (\%)} + 0.17 \times (p - 2.07)\], where \(p\) is plasma protein in g/dL.\(^{23, 24}\) Previously published plasma protein values from our laboratory (Luteal Phase: 6.6 g/dL,\(^{25}\) Third Trimester:\(^{26, 27}\) 6.2 g/dL) were used in the calculation. Shear stress was calculated as \(4 \times \text{viscosity} \times \text{shear rate}^{28}\).

**Data Analysis:** Brachial artery diameter was measured as described previously using automated edge-detection software (FMD/blood flow acquisition and analysis),\(^{22}\) which acquires continuous diameter measurements within a user-defined region of interest. Pre-inflation, and inflation, brachial artery diameter were defined as the median of all measurements obtained during the minute prior to cuff inflation, and the minute prior to cuff release, respectively. Peak diameter, and the time required to reach peak diameter, were determined by applying an automated algorithm\(^8\) to all measurements collected within two minutes of cuff release. The algorithm identifies peak diameter on a smoothed post-release diameter curve generated by calculating the median diameter during each time period from 100 data points, with an overlap of 20 data points for consecutive time periods.\(^8\) Blood flow velocity from the time of cuff release to the time of peak diameter was quantified using the same automated analysis program, which tracks the peak envelope of the velocity waveform within a user-defined region of interest.\(^8, 22\) Time aligned, two second averages for velocity and diameter from the time of cuff release until the time of peak dilation were computed, and shear rate for each two second period was calculated as
velocity/diameter. The shear rate or shear stress which may have contributed to diameter changes
during the period between cuff release and peak diameter was defined as the portion of reactive
hyperemia for which shear rate was 50% higher than the pre-inflation level. This always occurred
before peak FMD was achieved. Percent FMD normalized for shear rate or shear stress was
calculated as percent FMD/shear stimulus area under the curve (AUC). 13

In addition to determining FMD using true peak diameter as described above, FMD was
calculated using post-release diameter determined by four methods used in previous studies
examining the effects of pregnancy on FMD (Figure 4.1). 1-7 The 60 second (60s) method used by
Faber-Svensson and colleagues 2 defines post-release diameter as brachial artery diameter at 60
seconds post-release. The 55-65 second (55-65s) average method described by Savvidou and
colleagues 5 calculates post-release diameter as the average of all measurements between 55 and
65 seconds post-release. Three studies conducted on the same data set determined post-release
diameter at 40, 60 and 80 seconds post-release.3, 4, 6 While none of the studies state whether the
average of all measurements or the peak measurement was used to calculate FMD,3, 4, 6 the first
study 3 references a paper which used the maximum of the 40, 60 and 80 second measurements as
the post-release diameter. 29 Reported FMD is identical in all papers;3, 4, 6 hence it is likely that all
used the same method. The 40-60-80 second maximum (40-60-80s max.) method therefore
defines post-release diameter as the maximum of diameter measurements obtained at 40, 60 and
80 seconds post-release. Two studies 1, 7 measured post-release diameter as the average of
measurements from the first 4 cardiac cycles for which clear images were obtained, starting at 45
seconds post-release (I. Dorup, personal communication, A. Quinton, personal communication).
The 45 second method therefore calculates post-release diameter as the average of continuous
measurements obtained between 44 and 46 seconds. Post-release diameters determined by each of
Figure 4.1: Sample data from an active pregnant subject

Peak diameter occurred at 61 seconds. Gray circles represent raw data; black squares represent 2 second median (diameter) or average (velocity) of raw post-release data.
the 40-60-80s max., 45s, 60s, and 55-65s average methods were used to compute absolute and percent FMD, and results were compared with true peak FMD.

**Statistical Analysis:** The effects of chronic activity on gestational age, menstrual cycle day, weighted total activity, gestational age at delivery, and birth weight were determined using independent-samples t-tests. The effects of pregnancy and activity on the odds of delivering a male infant, and parity, were examined using chi-squared tests. Comparisons of MVPA in active and inactive subgroups were performed using Wilcoxin’s Rank-Sum test. The effects of pregnancy and chronic activity on all remaining subject characteristics and physical activity indices were assessed using a 2 x 2 analysis of variance (ANOVA) with pregnancy (non-pregnant vs. pregnant) and activity (active vs. inactive) as between-subjects factors. Outliers were observed for the difference between pre-inflation and inflation diameter and FMD in one active pregnant subject. Image quality during occlusion was poor in two additional active non-pregnant subjects. These subjects were removed from all calculations of means, standard deviations, and analyses of variance (ANOVAs), however, they were displayed on graphs. Two active pregnant subjects were removed from regression analyses for FMD vs. shear rate AUC, and FMD vs. baseline diameter, due to high Cook’s distance and centered leverage values which indicated that these points were outliers and had disproportionate influence on the slope of the regression line, and strength of the correlation. Comparisons between active and inactive subgroups revealed no significant main effects of activity or interactions with activity for any outcome measures; therefore active and inactive groups were pooled. All variables were normally distributed. Differences in time required to reach true peak diameter, and shear rate, between pregnant and non-pregnant women were assessed using independent samples t-tests. The effect of baseline measurement time on artery diameter and shear rate were assessed using a 2 x 2 repeated
measures ANOVA with baseline measurement time as the within-subjects factor (pre-inflation vs. inflation), and pregnancy as the between-subjects factor (pregnant vs. non-pregnant). Changes in heart rate and blood pressure during reactive hyperemia were assessed using a 3 x 2 repeated measures ANOVA, with time period as the within-subjects factor (pre-inflation baseline, inflation baseline, post-release) and pregnancy as the between-subjects factor. The effect of post-release measurement method on percent FMD was assessed using a 5 x 2 repeated measures ANOVA with method as the within-subjects factor (true peak, 40-60-80s max., 45s, and 60s, 55-65s average) and pregnancy as the between-subjects factor. The effects of pregnancy on differences between true peak FMD, and FMD calculated by each method, were evaluated using a 4 x 2 repeated measures ANOVA, with method (true peak – 40-60-80s max., true peak – 45s, true peak – 60s, true peak – 55-65s average) as the within-subjects factor, and pregnancy as the between-subjects factor. Where significant main effects were present, simple main effects were determined using paired or independent samples t-tests with the Sidak correction for multiple comparisons. Statistical significance for all analyses was determined by a two-sided p value < 0.05. All analyses were performed in SPSS 16.0 (SPSS Inc., Chicago, IL).

4.4 Results

Physical Characteristics of Subjects and Pregnancy Outcome: Gestational age at the time of the test did not differ between active and inactive pregnant women, and the cycle day on which the test was performed did not differ between active and inactive non-pregnant women (Table 4.1). Age was similar in all subject groups. BMI was significantly greater in pregnant women than in non-pregnant women of the same activity level, and was significantly increased in inactive women compared to active controls. Self-reported BMI prior to conception in pregnant women did not differ from BMI on the day of the exercise test in non-pregnant women. Active
women were more likely to be nulli/primiparous than inactive women, however the percentage of nulli/primiparous women did not differ between pregnant and non-pregnant subgroups of the same activity level.

Table 4.1: Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANP (n = 19)</th>
<th>INP (n = 11)</th>
<th>AP (n = 17)</th>
<th>IP (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.5 ± 4.7</td>
<td>33.3 ± 4.8</td>
<td>31.8 ± 3.6</td>
<td>30.3 ± 3.1</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>N/A</td>
<td>N/A</td>
<td>34.1 ± 1.3</td>
<td>34.2 ± 2.2</td>
</tr>
<tr>
<td>Menstrual Cycle Day</td>
<td>25 ± 2</td>
<td>23 ± 2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pre-conception BMI (kg/m²)</td>
<td>N/A</td>
<td>N/A</td>
<td>23.3 ± 3.0</td>
<td>26.2 ± 4.7</td>
</tr>
<tr>
<td>Test Day BMI (kg/m²)</td>
<td>22.5 ± 2.7</td>
<td>25.8 ± 4.4†</td>
<td>28.1 ± 3.2*</td>
<td>31.4 ± 6.2*‡</td>
</tr>
<tr>
<td>% Nulliparous/Primiparous</td>
<td>89%</td>
<td>36%†</td>
<td>76%</td>
<td>50%†</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations. Abbreviations: N/A, not applicable.

Significantly different from non-pregnant group: *p<0.01.
Significantly different from active group: †p<0.01, ‡p<0.05.

Low mean voluntary physical activity and Sports & Exercise Index scores confirm that inactive pregnant women had not been exercising regularly throughout pregnancy, and inactive non-pregnant women had not exercised regularly during the year prior to the study (Table 4.2). Contrast, the high scores obtained in active women confirm that active pregnant women exercised regularly throughout pregnancy and during the year prior to conception, whereas active non-pregnant women exercised regularly during the year prior to the study. Compared to inactive women, significant increases in mean voluntary physical activity and Sports & Exercise Index scores demonstrate that exercise participation was significantly greater in active women. The Active Living Index was also significantly increased among active non-pregnant women, and active pregnant women during pregnancy, compared to inactive controls. This indicates that
Table 4.2: Physical activity characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Current Activity</th>
<th></th>
<th>Chronic Activity Indices</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP</td>
<td>IP</td>
<td>ANP</td>
<td>INP</td>
</tr>
<tr>
<td>Daily Energy Expenditure (kcal)</td>
<td>3249 ± 597</td>
<td>3322 ± 606</td>
<td>2842 ± 580</td>
<td>3352 ± 998</td>
</tr>
<tr>
<td>Daily Energy Expenditure (kcal/kg/15 minutes)</td>
<td>42 ± 3</td>
<td>39 ± 2</td>
<td>46 ± 5</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Mean Voluntary Physical Activity (kcal)</td>
<td>264 ± 132</td>
<td>0 ± 0 †</td>
<td>529 ± 297</td>
<td>15 ± 41 †</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>AP</th>
<th>IP</th>
<th>ANP</th>
<th>INP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household and Family Care</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>2.5 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>Pregnant</td>
<td>2.3 ± 0.4 ‡</td>
<td>2.4 ± 0.3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Occupational</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>2.7 ± 0.8</td>
<td>2.7 ± 0.8</td>
<td>2.5 ± 0.6</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Pregnant</td>
<td>2.5 ± 0.8</td>
<td>2.8 ± 0.8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Active Living</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>3.4 ± 0.7</td>
<td>3.1 ± 0.5</td>
<td>3.7 ± 0.8</td>
<td>2.6 ± 0.5*</td>
</tr>
<tr>
<td>Pregnant</td>
<td>3.0 ± 0.4 ‡</td>
<td>2.3 ± 0.6 †;‡</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Sports and Exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>4.4 ± 0.4</td>
<td>2.6 ± 1.3*;§</td>
<td>4.3 ± 0.4</td>
<td>1.5 ± 0.6†</td>
</tr>
<tr>
<td>Pregnant</td>
<td>4.1 ± 0.3</td>
<td>1.4 ± 0.5†;‡</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Total Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception/Non-Pregnant</td>
<td>12.9 ± 1.1</td>
<td>10.9 ± 1.4*;§</td>
<td>12.7 ± 1.3</td>
<td>8.9 ± 1.3 †</td>
</tr>
<tr>
<td>Weighted Total Activity</td>
<td>10.4 ± 1.3</td>
<td>9.6 ± 0.8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Current activity data were available for 7 out of 11 INP subjects, whereas Chronic Activity Indices were available for 9 out of 11 INP subjects. Abbreviations: N/A, not applicable.
Significant difference from non-pregnant group of same activity level: *p<0.01.
Significant difference from active control group of same reproductive status: †p<0.01.
Significant difference from preconception: ‡p<0.01, §p<0.05.
active women spent more time engaged in active transportation (walking/cycling to and from work, school, or errands), and less time watching television.

Pregnancy outcome data were not available for one active pregnant subject who delivered at home. One inactive pregnant subject developed gestational hypertension, and delivered a healthy infant at 37.7 weeks gestation. All other women had uncomplicated pregnancies and delivered healthy infants after 37 weeks gestation. Gestational age at delivery (mean ± SD, Active: 39.9 ± 1.2 weeks, Inactive: 39.5 ± 1.3 weeks), birth weight (Active: 3616 ± 336g, Inactive: 3541 ± 292g), and the odds of delivering a male infant (Active: 40%, Inactive: 44%) did not differ between active and inactive pregnant groups.

**Effect of Pregnancy and Activity on Heart Rate and Blood Pressure Responses to Reactive Hyperemia:**

Heart rate during reactive hyperemia was significantly increased in pregnant women compared to non-pregnant controls (Table 4.3, p<0.001). There was no main effect of baseline measurement time (p=0.256), and no interaction between measurement time and pregnancy (p=0.144). Systolic and diastolic finger arterial pressure did not differ significantly between pregnant and non-pregnant women (p=0.277 and 0.904, respectively, for main effects). There were very small (3 mmHg), but statistically significant, increases in systolic and diastolic pressure during inflation (p<0.01 for main effects). Compared to measurements during inflation, systolic pressure did not change following cuff release, whereas diastolic pressure returned to pre-inflation levels post-release.
Table 4.3: Heart rate and blood pressure during reactive hyperemia in pregnant and non-pregnant women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Non-Pregnant (n = 28)</th>
<th>Pregnant (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (beats/min)</td>
<td>Pre-inflation</td>
<td>68 ± 10</td>
<td>89 ± 12(^i)</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>70 ± 9</td>
<td>87 ± 12(^i)</td>
</tr>
<tr>
<td></td>
<td>Post-Release</td>
<td>68 ± 10</td>
<td>87 ± 12(^i)</td>
</tr>
<tr>
<td>Systolic Finger Arterial Pressure (mmHg)</td>
<td>Pre-inflation</td>
<td>125 ± 18(^\dagger)</td>
<td>119 ± 18(^f)</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>128 ± 17</td>
<td>122 ± 19</td>
</tr>
<tr>
<td></td>
<td>Post-Release</td>
<td>127 ± 16</td>
<td>121 ± 17</td>
</tr>
<tr>
<td>Diastolic Finger Arterial Pressure (mmHg)</td>
<td>Pre-inflation</td>
<td>64 ± 10(^*)</td>
<td>64 ± 11(^i)</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>67 ± 9</td>
<td>67 ± 11</td>
</tr>
<tr>
<td></td>
<td>Post-Release</td>
<td>64 ± 8(^*)</td>
<td>65 ± 10(^*)</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations.
Significant difference from Inflation: \(^*p<0.01\), \(^\dagger p<0.05\).
Significant difference from Non-pregnant group: \(^i p<0.01\).

**Effect of Pregnancy, Activity and Cuff Inflation on Diameter and Shear Rate:** Analyses of the effects of cuff inflation on diameter and shear rate revealed no significant main effects of activity, or interactions with activity (Figure 4.2). When active and inactive women were pooled, baseline diameter was greater in pregnant than in non-pregnant women (Panel A; Main effect of pregnancy: \(p=0.016\)), and decreased slightly, but significantly, during inflation only in pregnant women (cuff inflation x pregnancy: \(p<0.001\)). Pre-inflation shear rate was greater in pregnant women than in non-pregnant controls (Panel B; Main effect of pregnancy: \(p<0.001\)), and was reduced to very low levels during cuff inflation that were not different between groups (Main effect of inflation: \(p<0.001\), pregnancy x inflation: \(p<0.001\)).
Figure 4.2: Effects of occlusion on brachial artery diameter and shear rate in pregnant and non-pregnant women

Solid lines represent active subjects. Dashed lines represent inactive subjects. Grey circles indicate the group mean, pooled for activity. Active and inactive groups were pooled for analysis as there were no main effects of activity. Significant difference from non-pregnant group at same timepoint: *p ≤ 0.001. Significant difference from pre-inflation: †p ≤ 0.004. Significant difference from non-pregnant group pre-inflation: ‡p=0.001.
Effect of Pregnancy and Activity on Brachial Artery Responses to Reactive Hyperemia:

There were no significant main effects of activity, interactions between pregnancy and activity, on absolute or percent FMD, the diameter change from pre-inflation to inflation, shear rate AUC, or time to peak diameter. Active and inactive women were therefore pooled for these analyses. Absolute FMD (p=0.973), percent FMD (p=0.194), and relevant shear rate AUC (p=0.137) did not differ significantly between pregnant and non-pregnant women (Table 4.4). There was a slight vasoconstriction during cuff inflation among pregnant women (-1.9 ± 3.1%) which was not observed in non-pregnant women (0.4 ± 3.6%), resulting in a significant difference in the diameter change during occlusion (p=0.021). Time to peak diameter was significantly increased among pregnant women compared to non-pregnant controls (p=0.012).

Table 4.4: Effect of pregnancy on brachial artery FMD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-Pregnant (n = 28)</th>
<th>Pregnant (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter Change from Pre-</td>
<td>0.4 ± 3.6</td>
<td>-1.9 ± 3.1*</td>
</tr>
<tr>
<td>inflation to Inflation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD (mm)</td>
<td>0.317 ± 0.116</td>
<td>0.327 ± 0.134</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>10.7 ± 3.8</td>
<td>9.6 ± 3.8</td>
</tr>
<tr>
<td>Shear Rate (AUC, s⁻¹)</td>
<td>9700 ± 3157</td>
<td>11275 ± 4075</td>
</tr>
<tr>
<td>Time to Peak Diameter (s)</td>
<td>46 ± 16</td>
<td>57 ± 15*</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations. Abbreviations: AUC, area under curve.
Significantly different from non-pregnant group: *p<0.05.

Relationships Between Pre-inflation Diameter, Percent FMD, and Shear Rate AUC: There were no significant correlations between the excess in relevant shear rate above pre-inflation
baseline levels and brachial artery diameter in active (Figure 4.3, panel A) or inactive (Panels B) groups. Consistent with this, there was also no relationship between brachial artery diameter and percent FMD in active (Panel C) or inactive (Panel D) groups. The excess in relevant shear rate above pre-inflation baseline levels was positively correlated with percent FMD in active groups (Panel E), but not in inactive groups (Panel F).

**Effect of Pregnancy on Viscosity and Shear Stress:** In a subset of five pregnant and seven non-pregnant participants, the mean value of viscosity was lower in pregnant women than in non-pregnant women, although this difference did not achieve the p=0.05 level selected for statistical significance (5.52 ± 0.33 vs. 5.10 ± 0.36, p=0.064). Shear stress decreased significantly during inflation in both pregnant (20.3 ± 5.1 vs. 5.6 ± 1.4, p<0.001) and non-pregnant women (23.3 ± 4.9 vs. 3.2 ± 1.2, p<0.001), however did not differ significantly between pregnant and non-pregnant groups prior to or during inflation (p=0.830 for main effect). The relevant shear stress AUC did not differ between pregnant (2113 ± 429) and non-pregnant (2311 ± 715) women (p=0.596).

**Effect of Method on FMD:** Peak percent FMD was greater than percent FMD determined by all other methods in pregnant and non-pregnant women (Table 4.5, Main effect of method: p<0.001). Percent FMD determined by the 40-60-80s max. method was greater than percent FMD determined by the 60s and 55-65s average methods among non-pregnant women, and 45s method among pregnant women (Method x pregnancy: p=0.022, Main effect of pregnancy: p=0.492). Among non-pregnant women, the difference between percent FMD calculated using true peak diameter, and percent FMD calculated using true peak and 40-60-80s max. methods, were significantly less than the differences between true peak FMD and FMD calculated by the 60s and 55-65s methods (Main effect of method: p<0.001). Among pregnant women, the differences
Figure 4.3: Relationships between pre-inflation brachial artery diameter, percent FMD, and shear rate increase during reactive hyperemia

○ Active Pregnant; ● Active non-pregnant; □ Inactive pregnant; ■ Inactive non-pregnant. Dashed regression line represents pregnant group, and solid regression line represents non-pregnant group. Large circles/squares with error bars represent group means, and standard deviations. *p<0.05, †p=0.059.
Figure 4.3 continued: Relationships between pre-inflation brachial artery diameter, percent FMD, and shear rate increase during reactive hyperemia

○ Active Pregnant; ● Active non-pregnant; □ Inactive pregnant; ■ Inactive non-pregnant. Dashed regression line represents pregnant group, and solid regression line represents non-pregnant group. Large circles/squares with error bars represent group means, and standard deviations. *p<0.05, †p=0.059.
in percent FMD between the true peak and 45s method were significantly less than the differences between the true peak and 40-60-80s max. method. Percent FMD calculated using the 60s and 55-65s methods underestimated FMD to a greater extent in non-pregnant (Figure 4.4, Panels B, D) than in pregnant women (Method x pregnancy: p=0.032, Panels A, C).

Table 4.5: Effect of post-release diameter measurement method in FMD in pregnant and non-pregnant women

<table>
<thead>
<tr>
<th>Method</th>
<th>FMD (%)</th>
<th>Difference from True Peak Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Pregnant (n = 28)</td>
<td>Pregnant (n = 24)</td>
</tr>
<tr>
<td>True peak</td>
<td>10.7 ± 3.8</td>
<td>9.6 ± 3.8</td>
</tr>
<tr>
<td>40-60-80s max.</td>
<td>9.6 ± 3.7*</td>
<td>8.6 ± 3.6*</td>
</tr>
<tr>
<td>45s</td>
<td>9.0 ± 3.5*</td>
<td>7.6 ± 3.5*;†</td>
</tr>
<tr>
<td>60s</td>
<td>8.0 ± 4.0*;†</td>
<td>8.0 ± 4.2*</td>
</tr>
<tr>
<td>55-65s average</td>
<td>8.1 ± 3.7*;†</td>
<td>8.0 ± 4.3*</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. Abbreviations: N/A, not applicable. Significant difference from true peak diameter method: *p<0.01. Significant difference from 40-60-80 second maximum method, †p<0.01, ‡p<0.05. Significant difference from Non-pregnant group: §p<0.01.

Among non-pregnant women, the difference between percent FMD calculated using true peak diameter, and percent FMD calculated using true peak and 40-60-80s max. methods, were significantly less than the differences between true peak FMD and FMD calculated by the 60s and 55-65s methods (Main effect of method: p<0.001). Among pregnant women, the differences in percent FMD between the true peak and 45s method were significantly less than the differences between the true peak and 40-60-80s max. method. Percent FMD calculated using the
Figure 4.4: Effect of analysis method on post-release diameter during reactive hyperemia

Lines represent individual subjects; grey circles represent group mean. When active and inactive subjects are pooled, the 60s and 55-65s average methods underestimate FMD to a significantly greater extent in non-pregnant than in pregnant women (p<0.01, Table 4.5).
60s and 55-65s methods underestimated FMD to a greater extent in non-pregnant (Figure 4.4, Panels B, D) than in pregnant women (Method x pregnancy: p=0.032, Panels A, C).

4.5 Discussion
This is the first study to assess the effects of pregnancy on true peak brachial artery FMD, to account for physical activity and potential pregnancy-induced changes in the shear stimulus and time course of peak dilation, and to account for menstrual cycle effects in the control group. The results reveal three important physiological findings. First, decreases in vascular tone due to increased shear stress contribute minimally to increases in baseline brachial artery diameter during pregnancy. Second, when women at 30-36 weeks gestation are compared to non-pregnant women in the mid-late luteal phase, true peak brachial artery FMD, and the shear stimulus for FMD, do not differ between pregnant and non-pregnant women. The timing of peak dilation, however, is delayed in pregnancy. Third, neither the shear stimulus nor brachial artery FMD were affected by regular physical activity in pregnant or non-pregnant women. However, activity may alter the strength of the relationship between FMD and shear rate AUC.

Impact of Pregnancy on Brachial Artery Diameter: Previous studies have hypothesized that increases in brachial artery diameter during pregnancy\(^1, 5\) may be caused by shear mediated vasodilation.\(^1\) In accordance with this hypothesis, baseline shear rate is elevated in pregnant women compared to non-pregnant controls. However, substantial reductions in shear rate during 5 minutes of distal cuff inflation only decreased brachial artery diameter by \(-1.9 \pm 3.1\%\) in pregnant women, and inflation diameter was still 12% greater than pre-inflation diameter in non-pregnant controls (p<0.001). This suggests that pregnancy-induced increases in conduit artery diameter are primarily mediated by other mechanisms. Decreased sympathetic activity is unlikely to explain pregnancy-induced increases in brachial artery diameter, as muscle sympathetic nerve
activity is increased in normotensive pregnant women at 35 ± 0.6 weeks gestation compared to post-partum measurements, or non-pregnant controls.\textsuperscript{30} Therefore, pregnancy-induced increases in brachial artery diameter are likely due to structural remodeling.\textsuperscript{31}

**FMD and its stimulus are not enhanced in pregnancy:** True peak FMD, and the shear stimulus for FMD, did not differ between non-pregnant controls in the mid to late luteal phase and pregnant women at 30-36 weeks gestation. The relationship between FMD and shear rate AUC was also preserved during pregnancy in active women, as regression lines were nearly identical in pregnant and non-pregnant subgroups. Shear stress is the stimulus for endothelium-dependent FMD, however shear rate is frequently used as a surrogate index because viscosity is unlikely to change during an 8-minute reactive hyperemia trial (Shear stress = 4\*shear rate\*viscosity).\textsuperscript{28}

Increases in shear rate above pre-inflation levels during reactive hyperemia did not differ between pregnant and non-pregnant women, despite pregnancy-induced increases in artery diameter. Thus, shear rate may not be an appropriate index of shear stress for comparisons between pregnant and non-pregnant women. Pregnancy-induced decreases in viscosity\textsuperscript{32} could cause shear rate to systematically overestimate shear stress in pregnant women, relative to non-pregnant controls. Pre-inflation shear stress, and shear stress AUC, were determined using viscosity calculated from measured hematocrit in a subset of 5 pregnant and 7 non-pregnant women to investigate this potential limitation. Values were similar in healthy pregnant and non-pregnant women, suggesting that decreases in viscosity during late gestation were too small to cause a systematic bias when shear rate was used as an index of shear stress. Nevertheless, although shear rate provided an acceptable approximation of shear stress in the present study, larger studies are required to confirm this conclusion, and examine the relationship between shear rate and shear
stress at earlier stages of pregnancy. These results suggest that flow-mediated responsiveness to increased shear is preserved despite altered hemodynamic conditions in pregnancy.

The observation that true peak FMD was not altered in pregnancy is consistent with three data sets reporting no change in FMD at 28-35 weeks gestation,\textsuperscript{3-7} but contrasts with two studies reporting significant increases in FMD of 5.4\% at 32 weeks gestation,\textsuperscript{2} and 3.4\% at 35 weeks gestation.\textsuperscript{1} These studies likely underestimated FMD in non-pregnant women, and overestimated the effect of pregnancy, due to two factors. First, neither study controlled for menstrual cycle phase.\textsuperscript{1,2} Two longitudinal studies demonstrated that FMD varies during the menstrual cycle in healthy young women.\textsuperscript{10, 11} The first observed increases in FMD during the late follicular (18.2 ± 0.8\%) and luteal (17.5 ± 0.7\%) phases compared to the early follicular (11.2 ± 0.6\%) phase.\textsuperscript{10} The second reported significant decreases in FMD during the early luteal phase (4.2 ± 0.6\%) compared to the early follicular (8.8 ± 0.6\%), late follicular (10.0 ± 0.7\%), and late luteal (8.6 ± 0.9\%) phases.\textsuperscript{11} While the reason for these contrasting results is unclear, both studies suggest that the inclusion of women in multiple phases in previous studies\textsuperscript{1-6} would have decreased percent FMD among non-pregnant women, increasing the magnitude of the difference between pregnant and non-pregnant women. Second, previous studies did not control for delayed peak dilation during pregnancy (57 ± 15 seconds vs. 46 ± 16 seconds) by using continuous measurements to identify true peak diameter. The 60s and 55-65s average methods underestimate percent FMD to a significantly greater extent in non-pregnant than in pregnant women (2.6-2.7\% vs. 1.6\%), as post-release diameter is measured near peak in pregnant women, but after diameter has declined in non-pregnant women. When combined with the inclusion of non-pregnant women in multiple menstrual cycle phases, this underestimation bias may contribute to erroneous conclusions that FMD is increased in pregnancy. In accordance with this hypothesis, the study which used the 60s
method reported the lowest FMD in non-pregnant women, and the largest difference between pregnant and non-pregnant women (Non-pregnant: 5.8 ± 2.1%, 32 weeks: 11.2 ± 5.5%). The significant pregnancy-induced increase in FMD observed by Dorup and colleagues (Non-pregnant: 7.2 ± 2.8%, 35 weeks: 10.6 ± 4.4%) is likely due to menstrual cycle phase, rather than underestimation bias. Dorup and colleagues used the 45s method, which reduced FMD by 1.8% and 2.1% in non-pregnant and pregnant women, respectively, in the present data set.

**Shear-FMD Relationship and Activity Level:** Regular physical activity did not alter the shear stimulus or brachial artery FMD in healthy non-pregnant and pregnant women. Larger studies would be beneficial to confirm these results, as the sample size of the inactive groups was lower than that of the active groups in the present study. 10 and 28 subjects per group would be required to detect significant differences of 5% and 3%, respectively, in FMD between active and inactive women using an independent samples t-test. FMD and shear rate AUC were positively correlated in active, but not inactive, pregnant and non-pregnant women, suggesting that physical activity alters the strength of the relationship between FMD and shear rate AUC. Larger studies are required to determine whether this reflects a real physiological effect, or was due to the smaller sample size in inactive groups. Studies should also determine whether this differential effect of activity may explain why FMD and shear rate AUC were correlated in active young men, but not in studies which have not accounted for physical activity.

**Conclusions:** Pregnancy-induced increases in brachial artery diameter are not a result of elevated shear-mediated dilation, but are likely due to structural remodeling. FMD, and the shear stimulus for FMD during a reactive hyperemia test, are not altered by the hemodynamic conditions of pregnancy at 30-36 weeks gestation, and this is independent of activity level. However, activity
level may influence the strength of the association between FMD and shear, in both pregnant and non-pregnant women. Finally, it is critical to identify true peak FMD with continuous diameter measurements, as other methods consistently underestimate FMD.
4.6 References


Chapter 5
Low Flow-Mediated Constriction Occurs in the Radial, but not Brachial, Artery in Healthy Pregnant and Non-pregnant Women

5.1 Abstract

Low flow-mediated vasoconstriction (L-FMC) during distal occlusion was recently proposed as a vascular health indicator. Recommendations that L-FMC be measured concurrently with flow-mediated dilation (FMD) were based on radial artery data. However, brachial artery FMD predicts cardiovascular disease; and therefore, it is important to determine whether L-FMC occurs in the brachial artery. The effects of pregnancy and physical activity on L-FMC are not known. Brachial and radial artery L-FMC and FMD were assessed in active non-pregnant (n=17), inactive non-pregnant (n=10), active pregnant (n=15, 34.1±1.2 weeks gestation), and inactive pregnant (n=8, 34.2±2.2 weeks gestation) women. Radial artery diameter decreased significantly during occlusion in all groups. Brachial artery diameter did not change in active and inactive non-pregnant, and inactive pregnant women, however, the small decrease in active pregnant women was significant. L-FMC occurs in the radial (Non-pregnant: 4.4 ± 4.2%, Pregnant: 6.4 ± 3.2%), but not the brachial artery, among non-pregnant and pregnant women. Radial artery L-FMC was not affected by activity or pregnancy. Positive correlations between L-FMC and FMD suggest that diameter may not return to pre-inflation values immediately after cuff release, and that FMD may measure hyperemia-induced dilation, superimposed on the normalization of factors regulating L-FMC following removal of the low-shear stimulus.

Keywords: low flow-mediated constriction, pregnancy, brachial artery, radial artery, physical activity
5.2 Introduction

Pregnant women experience significant remodeling of the cardiovascular system, including 30% increases in blood volume, 50% increases in cardiac output, 30% decreases in peripheral vascular resistance, and 20% decreases in mean arterial pressure. These hemodynamic changes could alter the mechanical (shear stress, transmural pressure) environment experienced by the vascular endothelium, which, in combination with hormonal alterations, might be expected to effect conduit artery structure and function. Brachial artery diameter and shear rate are increased during pregnancy, however, true peak flow-mediated dilation (FMD) does not differ between non-pregnant women in the mid-late luteal phase, and pregnant women at 30-36 weeks gestation (Chapter 2). These results suggest that hemodynamic changes modify conduit artery structure without effecting vascular function. However, FMD only measures vascular responsiveness to increased shear stress, and other aspects of conduit artery vascular function could be affected by pregnancy.

The degree of radial artery vasoconstriction during reduced flow caused by wrist occlusion (low flow-mediated constriction, L-FMC) has recently been proposed as a vascular health indicator. Radial artery L-FMC is diminished in hypertensive patients compared to healthy, young subjects. Radial artery L-FMC may be mediated through a combination of endothelin-1, cyclooxygenase products, and an endothelium-derived hyperpolarizing factor (EDHF), as L-FMC is attenuated by the endothelin-1 Type A receptor antagonist BQ-123, aspirin, and fluconazole.

Previous studies proposed that L-FMC should be measured concurrently with FMD. These recommendations were based on radial artery data, however, the radial artery is not traditionally
used for FMD assessment. Studies demonstrating that FMD predicts cardiovascular events in healthy individuals, middle-aged patients with no history of heart disease, elderly patients, and coronary artery disease patients examined brachial artery FMD following forearm occlusion. If L-FMC is to be measured concurrently with FMD, then it is important to determine whether L-FMC occurs in the brachial artery during the traditional FMD protocol. The effect of artery on L-FMC has not been systematically examined, however, available data suggest that L-FMC in healthy subjects may be artery dependant. Three studies reported mean radial artery L-FMC values of 4-7% among healthy young and middle aged subjects during 4.5-5 minutes of wrist occlusion, however, results in the brachial artery are inconsistent. Investigators reported changes in mean brachial artery diameter of 2.4%, 10 -1.1%, 11 -1.7%, 12 and -15% 13 during 5 minutes of forearm occlusion in healthy young men and women. In middle-aged men and women, brachial artery diameter did not change during 5 minutes of forearm occlusion in healthy non-smokers, or during 5 minutes of wrist occlusion in normocholesterolemic control subjects. These results suggest that L-FMC occurs in the radial, but not the brachial artery in healthy subjects, however, paired measurements are required to confirm this hypothesis.

Previous studies also proposed that the sum of the absolute values of L-FMC and FMD may provide a more complete assessment of vascular function by incorporating responsiveness to both decreases and increases in shear stress from baseline levels. This interpretation of L-FMC and FMD as separate indicators is based on the assumption that L-FMC has fully reversed before peak FMD occurs. This assumption has not been tested. If the factors regulating L-FMC do not return to pre-inflation values before peak FMD, then FMD itself may be a composite of hyperemia-induced dilation, superimposed on the normalization of factors regulating L-FMC following removal of the low-shear stimulus. There was no relationship between radial artery L-
FMC and FMD in a previous study, supporting the conclusion that L-FMC and FMD provide independent information about endothelial function. Additional studies are needed to confirm this finding, and to examine the relationship between L-FMC and FMD in diverse populations.

The objectives of the present study were to examine the effects of pregnancy and physical activity on L-FMC, to determine whether L-FMC is artery-specific in healthy pregnant and non-pregnant women, to assess the relationship between L-FMC and FMD, and to determine whether pregnancy or physical activity modify the relationship between L-FMC and FMD. It was hypothesized that L-FMC occurs in both the brachial and radial arteries, that pregnancy and activity do not affect the magnitude of L-FMC, and that there would be no relationship between L-FMC and FMD.

5.3 Methods
Participants were 15 active pregnant (AP), 8 inactive pregnant (IP), 17 active non-pregnant (ANP), and 10 inactive non-pregnant (INP) women who completed a larger study examining the effects of pregnancy, and acute and chronic exercise on endothelial function. All women were healthy, non-smokers between the ages of 20 and 40, with no history of pregnancy-induced hypertension, who were not taking hormonal contraception or other medications. Pregnant women were tested between 30 and 36 weeks gestation (34.2 ± 1.6 weeks), whereas non-pregnant women were in the mid-late luteal phase (days 20-28). Active women had exercised for three or more hours/week for at least six months, while inactive women did not regularly exercise at a sufficient intensity to break into a sweat. The study was approved by the Health Sciences Research Ethics Board at Queen’s University, and all subjects provided written informed consent prior to participating.
Pre-test Screening: Non-pregnant women completed a medical screening form (Physical Activity Readiness Questionnaire, http://www.csep.ca/forms.asp) to ensure that they were healthy with no contraindications to exercise. Pregnant women completed a similar standardized form (PARmed-X for Pregnancy, http://www.csep.ca/forms.asp) with their obstetrician or midwife to obtain medical clearance. An abdominal ultrasound was performed to ensure that the subject was having a normal, healthy pregnancy and the fetus was not small for gestational age. An obstetrician (G.A.L. Davies) provided final approval for testing in pregnancy after examining each screening form and ultrasound report.

Physical Activity Indices: Women completed a 3-day physical activity record\(^\text{17}\) on consecutive days (two weekdays, one weekend day) within 2 weeks of the test to evaluate current physical activity. Daily energy expenditure (DEE) and maximum voluntary physical activity (MVPA) were calculated as described previously.\(^\text{17}\)

Chronic physical activity was evaluated using the Kaiser Physical Activity Survey (KPAS), which was validated in non-pregnant\(^\text{18}\) and pregnant\(^\text{19}\) women. The KPAS was delivered once to non-pregnant subjects to assess activity during the past year, and twice to pregnant subjects to assess activity during pregnancy and for one year before conception. Physical activity indices for Household & Family Care, Occupational, Active Living, Sports & Exercise, and Total Activity were calculated as described previously.\(^\text{18,19}\) Weighted total activity during pregnancy was computed by weighting each index by its average contribution to the energy expenditure of pregnant women (Household & Family Care: 50%, Occupational: 20%, Active Living: 25%, Sports & Exercise: 5%).\(^\text{19}\)
Endothelial function assessment: Women avoided caffeine and exercise for 12 hours before the test, and consumed a standard meal (350 kcal) at 7am. After arriving at the laboratory at 8am, women were seated in a semi-darkened room with the right arm resting on a table at the level of the heart. A child-sized blood pressure cuff attached to an automatic inflation device was wrapped around the extended right wrist and hand. Beat-to-beat blood pressure measurements were obtained using a Finapres (Model 2300, Ohmeda) photoplethysmographic cuff placed around the left middle finger. Continuous, grayscale images of the radial artery were obtained using a 10 MHz probe operating in B-Mode (GE Vingmed System 5, GE Medical Systems). Simultaneous blood flow velocity measurements were recorded using Doppler ultrasound, with the same probe operating at 4 MHz. An insonation angle of 68° was maintained throughout each trial, to allow for accurate velocity measurements while optimizing image quality by ensuring that the ultrasound beam was perpendicular to the vessel.20 The probe was positioned over the radial artery between 10 and 15 centimeters proximal to the cuff, wherever the clearest image was obtained. Baseline data were recorded for one minute, after which the occlusion cuff was inflated to 250 mmHg for 5 minutes. Measurements were collected for two minutes post-release.

The left arm was then positioned on a table at the level of the heart. A large blood pressure cuff attached to an automatic inflation device was placed on the forearm distal to the elbow. The ultrasound probe was positioned over the brachial artery between 2 and 8 centimeters proximal to the cuff, wherever the clearest image was obtained. Resting brachial artery diameter was determined during a one-minute scan, after which the occlusion cuff was inflated to 250 mmHg for 5 minutes. Measurements were collected for two minutes post-release. The right arm was repositioned on the table at the level of the heart. The radial artery reactive hyperemia protocol was then repeated.
During each trial, artery diameter and blood flow velocity measurements were collected during one minute before cuff inflation, the last minute before cuff release, and for two minutes following release (Figure 5.1). Ultrasound images of brachial artery diameter and velocity were recorded on video tape, then transferred to a Digital Imaging and Communications in Medicine (DICOM) file at a rate of 25 frames per second for offline analysis.21

**Data Analysis:** Artery diameter was measured as described previously using automated edge-detection software (FMD/blood flow acquisition and analysis),21 which acquires continuous diameter measurements within a user-defined region of interest. Pre-inflation, and inflation, artery diameters were defined as the median of all measurements obtained during the minute prior to cuff inflation, and the minute prior to cuff release, respectively. Peak diameter, and the time required to reach peak diameter, were determined by applying an automated algorithm22 to all measurements collected within two minutes of cuff release. The algorithm identifies peak diameter on a smoothed post-release diameter curve generated by calculating the median diameter during each time period from 100 data points, with an overlap of 20 data points for consecutive time periods.22 Blood flow velocity from the time of cuff release to the time of peak diameter was quantified using the same automated analysis program, which tracks the peak envelope of the velocity waveform within a user-defined region of interest.21,22 Time aligned, two second averages for velocity and diameter from the time of cuff release until the time of peak dilation were computed. Shear rate (a measure of the shear stimulus without accounting for viscosity), was calculated as velocity/diameter for each two second period.
Figure 5.1: Sample data from the radial artery of an active pregnant subject

Peak diameter occurred at 66 seconds. Gray circles represent raw data; black squares represent 2 second median (diameter) or average (velocity) of raw post-release data.
**Statistical Analysis:** Change in mean, typical error (the within-subjects, standard deviation, after accounting for changes in the mean), and intraclass correlation coefficients of radial artery diameter and L-FMC measurements between trials 1 and 2 were calculated using a spreadsheet program (Reliability spreadsheet, available at www.sportsstats.org). A one sample t-test was used to determine whether the change in the mean diameter and L-FMC between trials 1 and 2 was significantly different from 0.

The effects of chronic activity on gestational age, menstrual cycle day, weighted total activity, gestational age at delivery, and birth weight were determined using independent-samples t-tests. The effects of pregnancy and activity on the odds of delivering a male infant, and parity, were examined using chi-squared tests. Comparisons of MVPA in active and inactive subgroups were performed using Wilcoxin’s Rank-Sum test. The effects of pregnancy and chronic activity on all remaining subject characteristics and physical activity indices were assessed using a 2 x 2 analysis of variance (ANOVA) with pregnancy (non-pregnant vs. pregnant) and activity (active vs. inactive) as between-subjects factors.

Two active non-pregnant subjects were removed from calculations of means, standard deviations, and analyses of variance (ANOVAs) for comparisons between the brachial and radial arteries due to poor image quality in the brachial artery during occlusion and post-cuff release. Data from these subjects was displayed on graphs. All variables were normally distributed. The effects of pregnancy on subject characteristics, radial artery L-FMC, shear rate AUC, and time to peak diameter were assessed using independent samples t-tests. Radial artery measurements were the average of values obtained in reactive hyperemia trials 1 and 2. The effects of pregnancy, activity, artery (brachial vs. radial), and cuff inflation (pre-inflation vs. inflation) were assessed using
repeated measures ANOVA, with artery and cuff inflation as within-subjects factors, and activity and pregnancy as between-subjects factors. In cases where comparisons between active and inactive subgroups revealed no significant main effects of activity or interactions with activity, active and inactive groups were pooled. Where significant main effects were present, simple main effects were determined using paired or independent samples t-tests with the Sidak correction for multiple comparisons. Relationships between L-FMC, FMD, and changes in shear rate in the brachial and radial arteries were assessed using Pearson correlation coefficients. High Cooks distance and centered leverage values were used to identify outliers which had a disproportionate influence on the slope of the regression line, and strength of the correlation. These points were excluded from regression analyses, but are shown on graphs. Statistical significance for all analyses was determined by a two-sided p value < 0.05. All analyses were performed in SPSS 16.0 (SPSS Inc., Chicago, IL).

5.4 Results

Physical Characteristics and Activity Participation: Age did not differ between groups (Table 5.1). Gestational age was similar in active and inactive pregnant women, while the cycle day on which testing occurred did not differ between active and inactive non-pregnant women. Inactive women were less likely to be nulliparous, however the magnitude of this was similar in active and inactive subgroups. BMI prior to conception in pregnant women was similar to BMI at the time of the exercise test in non-pregnant women, however BMI at the time of the exercise test was significantly increased in pregnant women.

High mean voluntary physical activity and Sports & Exercise Index scores confirmed that active pregnant women exercised regularly throughout pregnancy and the year prior to conception,
Table 5.1: Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active Non-pregnant (n = 17)</th>
<th>Inactive Non-pregnant (n = 10)</th>
<th>Active Pregnant (n = 15)</th>
<th>Inactive Pregnant (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.9 ± 4.6</td>
<td>32.7 ± 4.7</td>
<td>31.8 ± 3.6</td>
<td>30.3 ± 3.1</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Menstrual Cycle Day</td>
<td>25 ± 2</td>
<td>23 ± 3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>N/A</td>
<td>N/A</td>
<td>23.3 ± 3.0</td>
<td>26.2 ± 4.7</td>
</tr>
<tr>
<td>Test Day BMI (kg/m²)</td>
<td>22.5 ± 2.8</td>
<td>25.8 ± 4.7</td>
<td>28.1 ± 3.2*</td>
<td>31.4 ± 6.2*</td>
</tr>
<tr>
<td>Parity (% nulliparous)</td>
<td>88%</td>
<td>40%†</td>
<td>77%</td>
<td>50%†</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations. Abbreviations: N/A, not applicable; BMI, body mass index.

Significantly different from non-pregnant group: *p<0.01.
Significantly different from active group: †p<0.01, ‡p=0.052-0.054.
Significantly different from preconception: §p<0.01.

whereas active non-pregnant women exercised regularly during the year prior to the study (Table 5.2). In contrast, low scores demonstrated that inactive pregnant women had not exercised regularly during pregnancy, and inactive non-pregnant women had not exercised regularly during the year prior to the study. Mean voluntary physical activity and Sports & Exercise Index scores were significantly increased in active women compared to inactive women, demonstrating that exercise participation was greater in active women.

Pregnancy Outcome: Outcome data were not available for one active pregnant participant who delivered at home. One inactive pregnant participant developed gestational hypertension, and delivered a healthy infant at 37.7 weeks gestation. All other participants delivered healthy infants after 37 weeks gestation. Gestational age at delivery (Active: 39.9 ± 1.1 weeks; Inactive: 39.5 ±
Table 5.2: Physical activity characteristics of subjects

<table>
<thead>
<tr>
<th>Current Activity</th>
<th>ANP</th>
<th>INP</th>
<th>AP</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Energy Expenditure (kcal)</td>
<td>2813 ± 589</td>
<td>3434 ± 1067</td>
<td>3249 ± 596</td>
<td>3322 ± 606</td>
</tr>
<tr>
<td>Daily Energy Expenditure (kcal/kg/15 minutes)</td>
<td>45 ± 4</td>
<td>45 ± 5</td>
<td>42 ± 3</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Mean Voluntary Physical Activity (kcal)</td>
<td>497 ± 276</td>
<td>18 ± 44†</td>
<td>264 ± 131</td>
<td>0 ± 0§</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chronic Activity Indices</th>
<th>ANP</th>
<th>INP</th>
<th>AP</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household and Family Care</td>
<td>2.2 ± 0.4</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.4</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Pregnant</td>
<td>N/A</td>
<td>N/A</td>
<td>2.3 ± 0.4§</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Occupational</td>
<td>2.4 ± 0.5</td>
<td>2.1 ± 0.9</td>
<td>2.7 ± 0.8</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Pregnant</td>
<td>N/A</td>
<td>N/A</td>
<td>2.5 ± 0.8</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Active Living</td>
<td>3.6 ± 0.8</td>
<td>2.6 ± 0.5</td>
<td>3.4 ± 0.7</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Pregnant</td>
<td>N/A</td>
<td>N/A</td>
<td>3.0 ± 0.4†</td>
<td>2.3 ± 0.6‡</td>
</tr>
<tr>
<td>Sports and Exercise</td>
<td>4.3 ± 0.4</td>
<td>1.6 ± 0.6‡</td>
<td>4.4 ± 0.4</td>
<td>2.6 ± 1.3*†</td>
</tr>
<tr>
<td>Pregnant</td>
<td>N/A</td>
<td>N/A</td>
<td>4.1 ± 0.3</td>
<td>1.4 ± 0.5†‡</td>
</tr>
<tr>
<td>Total Activity</td>
<td>12.5 ± 1.2</td>
<td>9.1 ± 1.2†</td>
<td>12.9 ± 1.1</td>
<td>10.9 ± 1.4*‡</td>
</tr>
<tr>
<td>Weighted Total Activity</td>
<td>N/A</td>
<td>N/A</td>
<td>10.4 ± 1.3</td>
<td>9.6 ± 0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Current activity data were available for 7 out of 10 INP subjects, whereas Chronic Activity Indices were available for 8 out of 10 INP subjects. Abbreviations: N/A, not applicable; ANP, active non-pregnant; INP, inactive non-pregnant; AP, active pregnant; IP, inactive pregnant.
Significant difference from non-pregnant group of same activity level: *$p<0.01$.

Significant difference from active control group of same reproductive status: †$p<0.01$.

Significant difference from preconception: ‡$p<0.01$, §$p<0.05$. 
1.3 weeks), birth weight (Active: 3562 ± 359g; Inactive: 3541 ± 292g), and the odds of delivering a female infant (Active: 57%; Inactive: 56%) did not differ between active and inactive women.

Effects of Pregnancy and Activity on Heart Rate and Blood Pressure Responses to Reactive Hyperemia: Heart rate did not differ between brachial and radial artery trials (Main effect: p=0.512). Heart rate was increased in pregnant women compared to non-pregnant controls (Main effect: p=0.012), however, pairwise comparisons revealed that these differences only reached statistical significance in active women (Table 5.3). Heart rate was also greater in inactive than in active women (Main effect: p=0.003). This difference was statistically significant for all pairwise comparisons in non-pregnant women, however, among pregnant women, statistically significant differences were only observed in the radial artery during inflation (p-values for remaining comparisons: 0.071-0.224). Systolic and diastolic blood pressure did not differ between the brachial and radial artery trials (Main effects: p=0.486, p=0.310), and were not effected by pregnancy (Main effects: p=0.967, p=0.697), or activity (Main effects: p=0.662, p=0.415). Heart rate (Main effect of measurement time: p<0.001), systolic (Main effect of measurement time: p=0.007) and diastolic (Main effect of measurement time: p<0.001) finger arterial pressure increased by 1-4 beats/min or mmHg during cuff inflation. In some cases these small differences were statistically significant (Table 5.3).

Reliability of Radial Artery Diameter and L-FMC: High quality radial artery images were obtained throughout both reactive hyperemia trials in 22 non-pregnant and 21 pregnant women. High intraclass correlation coefficients (0.91-0.95) and the absence of significant changes in diameter between trials in pregnant and non-pregnant subgroups indicated good reliability for pre-inflation and inflation diameter measurements (Table 5.4). Reliability was lower for L-FMC,
Table 5.3: Heart rate and blood pressure during reactive hyperemia in pregnant and non-pregnant women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Brachial Artery</th>
<th>Radial Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ANP (n = 17)</td>
<td>INP (n = 10)</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>Pre-inflation</td>
<td>65 ± 8*</td>
<td>79 ± 11*</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>68 ± 8</td>
<td>79 ± 10*</td>
</tr>
<tr>
<td></td>
<td>Post-Release</td>
<td>66 ± 9†</td>
<td>76 ± 9*</td>
</tr>
<tr>
<td>Systolic Finger Arterial Pressure (mmHg)</td>
<td>Pre-inflation</td>
<td>122 ± 17†</td>
<td>121 ± 17</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>125 ± 18</td>
<td>123 ± 15</td>
</tr>
<tr>
<td></td>
<td>Post-Release</td>
<td>126 ± 16</td>
<td>119 ± 12</td>
</tr>
<tr>
<td>Diastolic Finger Arterial Pressure (mmHg)</td>
<td>Pre-inflation</td>
<td>64 ± 10*</td>
<td>68 ± 10</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>67 ± 11</td>
<td>69 ± 9</td>
</tr>
<tr>
<td></td>
<td>Post-Release</td>
<td>65 ± 11†</td>
<td>65 ± 7†</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations. Abbreviations: ANP, active non-pregnant; INP, inactive non-pregnant; AP, active pregnant; IP, inactive pregnant.

Significant difference from Inflation: *p<0.01, †p<0.05.

Trend towards significant difference from post-release: ‡p=0.051.

Significant difference from Non-pregnant group: §p<0.01, ¶p<0.05.

Significant difference from Active group: ‥p<0.01, ′p<0.05.
as intraclass correlation coefficients were 0.56 in non-pregnant women, 0.87 in pregnant women, and 0.71 in the entire sample. L-FMC was significantly greater in trial 2 than in trial 1 among both pregnant (1.3%, p=0.016) and non-pregnant (1.8%, p=0.042) women.

**Table 5.4: Reliability of radial artery diameter and L-FMC**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reliability Measure</th>
<th>Non-pregnant (n = 22)</th>
<th>Pregnant (n = 21)</th>
<th>Pooled (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-inflation Diameter (mm)</strong></td>
<td>Change in Mean</td>
<td>-0.015 (-0.046, 0.017)</td>
<td>-0.055 (-0.102, 0.008)</td>
<td>-0.042† (-0.072, 0.012)</td>
</tr>
<tr>
<td></td>
<td>Typical Error</td>
<td>0.061 0.081</td>
<td>0.049, 0.082</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>(0.89, 0.98)</td>
<td>0.95 (0.064, 0.113)</td>
<td>(0.069, 0.099)</td>
</tr>
<tr>
<td><strong>Inflation Diameter (mm)</strong></td>
<td>Change in Mean</td>
<td>0.013 (-0.025, 0.051)</td>
<td>-0.033 (-0.078, 0.012)</td>
<td>-0.008 (-0.037, 0.020)</td>
</tr>
<tr>
<td></td>
<td>Typical Error</td>
<td>0.073 0.080</td>
<td>0.059, 0.099</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.92 (0.83, 0.96)</td>
<td>0.91 (0.81, 0.96)</td>
<td>0.92 (0.87, 0.95)</td>
</tr>
<tr>
<td><strong>L-FMC (mm)</strong></td>
<td>Change in Mean</td>
<td>0.028 (0.007, 0.062)</td>
<td>0.039* (0.019, 0.058)</td>
<td>0.034* (0.016, 0.052)</td>
</tr>
<tr>
<td></td>
<td>Typical Error</td>
<td>0.066 0.033</td>
<td>0.053, 0.089</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.39 (0.04, 0.67)</td>
<td>0.86 (0.68, 0.94)</td>
<td>0.76 (0.61, 0.86)</td>
</tr>
<tr>
<td><strong>L-FMC (%)</strong></td>
<td>Change in Mean</td>
<td>1.8 (0.4, 3.3)†</td>
<td>1.3 (0.5, 2.2)†</td>
<td>1.4 (0.5, 2.2)†</td>
</tr>
<tr>
<td></td>
<td>Typical Error</td>
<td>2.8 (2.2, 3.7)</td>
<td>1.4 (1.1, 2.0)</td>
<td>2.3 (1.9, 2.8)</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.56 (0.25, 0.77)</td>
<td>0.87 (0.70, 0.94)</td>
<td>0.71 (0.54, 0.82)</td>
</tr>
</tbody>
</table>

Values represent means (90% confidence limits). Typical error is the within-subject standard deviation. Abbreviations: ICC, intraclass correlation coefficient.

Significant difference from 0, as determined by one sample t-test: *p<0.01, †p<0.05.

**Effects of Pregnancy and Artery on Diameter and L-FMC**: Brachial artery diameter was significantly greater than radial artery diameter in all groups during the pre-inflation and inflation baseline periods (Table 5.5, p<0.001 for main effect and all comparisons). Pregnancy
Table 5.5: Effect of artery, pregnancy, activity and cuff inflation on artery diameter

<table>
<thead>
<tr>
<th>Artery</th>
<th>Timepoint</th>
<th>Active Non-pregnant (n = 15)</th>
<th>Inactive Non-pregnant (n = 10)</th>
<th>Active Pregnant (n = 16)</th>
<th>Inactive Pregnant (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.987 ± 0.319</td>
<td>2.862 ± 0.380</td>
<td>3.469 ± 0.396$\text{§}$</td>
<td>3.239 ± 0.165$\text{¶}$</td>
</tr>
<tr>
<td></td>
<td>Pre-inflation</td>
<td>3.003 ± 0.350</td>
<td>2.870 ± 0.371</td>
<td>3.398 ± 0.384$\text{‡,§}$</td>
<td>3.189 ± 0.204$\text{ǁ}$</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>1.980 ± 0.309*</td>
<td>1.992 ± 0.217*</td>
<td>2.176 ± 0.271*,$\text{,j}$</td>
<td>2.320 ± 0.302*,$\text{¶}$</td>
</tr>
<tr>
<td>Radial</td>
<td>Pre-inflation</td>
<td>1.912 ± 0.281*,$\text{†}$</td>
<td>1.872 ± 0.254*,$\text{†}$</td>
<td>2.022 ± 0.260*,$\text{†}$</td>
<td>2.178 ± 0.277*,$\text{¶,†}$</td>
</tr>
<tr>
<td></td>
<td>Inflation</td>
<td>2.910 ± 0.319</td>
<td>2.862 ± 0.380</td>
<td>3.469 ± 0.396$\text{§}$</td>
<td>3.239 ± 0.165$\text{¶}$</td>
</tr>
</tbody>
</table>

Values represent means ± standard deviation, in mm.

Significant difference from brachial artery at same timepoint: *p<0.01.

Significant difference from pre-inflation: †p<0.01, ‡p<0.05.

Significant difference from non-pregnant group of the same activity level: §p<0.01, ¶p<0.05.

Trend towards significant difference from non-pregnant control group of the same activity level: ‖p=0.057-0.060.
significantly increased pre-inflation brachial artery diameter (Main effect: p<0.001) among active and inactive women, inflation brachial artery diameter among active women, and pre-inflation and inflation radial artery diameter in active and inactive women, compared to non-pregnant controls of the same activity level. Increases in pre-inflation radial artery diameter among active pregnant women, and inflation brachial artery diameter among inactive pregnant women, did not reach statistical significance (p=0.057-0.060). Radial artery diameter decreased significantly during cuff inflation in all groups (p<0.001), however brachial artery diameter did not change during cuff inflation in active and inactive non-pregnant, and inactive pregnant women (Main effect of cuff inflation: p<0.001; cuff inflation x artery: p<0.001). There was a small (1.9%, p=0.014), but statistically significant, decrease in brachial artery diameter during cuff inflation in active pregnant women (cuff inflation x pregnancy: p=0.005; artery x activity: p=0.035). These results indicate the L-FMC occurred in the radial artery, but did not occur in the brachial artery.

Active and inactive women were pooled for analysis of L-FMC, as there were no main effects of activity, or significant interactions that included activity. Radial artery L-FMC was significantly lower than brachial artery L-FMC in both pregnant and non-pregnant women (Figure 5.2, Main effect of artery: p<0.001). L-FMC tended to be lower in pregnant than in non-pregnant women (Main effect of pregnancy: p=0.005), however this difference only reached statistical significance in the brachial artery (Brachial: p=0.022, Radial: p=0.082). Radial artery diameter decreased during cuff inflation in 84% and 96% of trials in non-pregnant and pregnant subjects, did not change in 4% of trials in pregnant subjects, and increased slightly in 16% of trials in non-pregnant subjects, respectively. In contrast, brachial artery diameter decreased during occlusion in 44% of non-pregnant and 71% of pregnant subjects, remained unchanged in 4% of non-pregnant and pregnant subjects, and increased in 52% of non-pregnant and 25% of pregnant subjects.
Figure 5.2: Brachial and radial artery L-FMC, and the relationship between the change in radial artery shear rate during occlusion and L-FMC in pregnant and non-pregnant women

A: Solid lines represent active subjects; dashed lines represent inactive subjects. Gray circles represent group means. B, C: ■ Active group; □ Inactive group. Small squares represent individual subjects; large squares represent group means, error bars are standard deviations. 90% confidence limits are approximately ±0.30.

Significant difference from non-pregnant group: *p<0.01
Significant difference from brachial artery: †p<0.05.
**Effects of Pregnancy and Artery on Shear Rate:** Active and inactive women were pooled, as there were no main effects of activity on shear rate, or significant interactions that included activity. Pre-inflation shear rate in the brachial and radial arteries was significantly greater in pregnant women than in non-pregnant controls, however shear rate during inflation did not differ between pregnant and non-pregnant women (Main effect of pregnancy: \( p<0.001 \), Pregnancy x cuff inflation: \( p<0.001 \)). Shear rate decreased significantly following cuff inflation in the brachial and radial arteries of pregnant and non-pregnant women (\( p<0.001 \) for main effect, and all pairwise comparisons). Pre-inflation shear rate did not differ between the brachial and radial arteries, however, shear rate during inflation was significantly greater in the radial than in the brachial artery (Main effect of artery: \( p=0.108 \); Cuff inflation x artery: \( p=0.047 \)). The percent reduction in shear rate during cuff occlusion was not significantly correlated with radial artery L-FMC in any subgroup, or among pregnant (\( r=0.19, p=0.381 \)) or non-pregnant women (\( r=0.10, p=0.613 \)) when active and inactive subgroups were pooled (Figure 5.2).

**Effects of Pregnancy and Artery on FMD, and the Combined Index:** There were no significant effects of activity on FMD, therefore activity and inactive women were pooled. Brachial and radial artery FMD did not differ between pregnant and non-pregnant women (Figure 5.3, Main effect of pregnancy: \( p=0.556 \)). Brachial artery FMD was significantly greater than radial artery FMD among non-pregnant (\( p=0.031 \)), but not pregnant (\( p=0.528 \)) women (Main effect of artery: \( p=0.051 \)). There was no effect of activity on the radial artery combined index (L-FMC + FMD), and values did not differ significantly between pregnant and non-pregnant women (\( p=0.084 \)). The brachial artery combined index was not calculated, as L-FMC did not occur.
**Relationship Between L-FMC and FMD:** There was a significant positive correlation between radial artery L-FMC and FMD in inactive non-pregnant (r=0.86, p=0.002), but not in active non-pregnant (r=0.35, p=0.167), active pregnant (r=-0.12, p=0.666) and inactive pregnant (r=0.48, p=0.233) women (Figure 5.3). Radial artery L-FMC and FMD were significantly correlated when non-pregnant (r=0.69, p<0.001) and all subject groups (r=0.45, p=0.001) were pooled, however not when pregnant women were pooled (r=0.25, p=0.248). Significant positive correlations between brachial artery L-FMC and FMD were observed among inactive non-pregnant (r=0.81, p=0.005), but not in active non-pregnant (r=0.21, p=0.417), active pregnant (r=0.04, p=0.888) and inactive pregnant (r=0.64, p=0.089) women. Brachial artery L-FMC and FMD were significantly correlated when non-pregnant (r=0.45, p=0.021), pregnant (r=0.42, p=0.047) and all groups (r=0.39, p=0.006) subjects were pooled.

**5.5 Discussion**

This is the first study to obtain paired brachial and radial artery L-FMC measurements, and to examine the effects of pregnancy and physical activity on L-FMC. The results reveal four important physiological findings. First, the occurrence of L-FMC in healthy pregnant and non-pregnant women is artery-dependant. L-FMC occurs in the radial artery during 5 minutes of wrist occlusion in most healthy non-pregnant and pregnant women, however, L-FMC does not occur in the brachial artery during 5 minutes of forearm occlusion. Second, positive correlations between L-FMC and FMD indicate that FMD is reduced among women who experience greater L-FMC, suggesting that factors influencing the magnitude of L-FMC are able to influence FMD. Third, when women at 30-36 weeks gestation are compared to non-pregnant subjects in the menstrual cycle phase most similar to pregnancy, radial artery L-FMC did not differ significantly between
Figure 5.3: Relationship between L-FMC and FMD in the brachial and radial arteries

Black squares, Active non-pregnant; Open squares, Active Pregnant; Black triangles, Inactive non-pregnant; Open triangles, Inactive Pregnant. Small squares represent individual subjects; large squares represent group means, error bars are standard deviations. *p<0.01, †p<0.05.

Pooled Non-pregnant: r² = 0.48*
Pooled pregnant: r² = 0.06
ANP: r² = 0.12, p=0.167
INP: r² = 0.74*

Pooled Non-pregnant: r² = 0.20†
Pooled pregnant: r² = 0.18†
ANP: r² = 0.04, p=0.417
INP: r² = 0.66*
Figure 5.3 continued: Relationship between L-FMC and FMD in the brachial and radial arteries

Black squares, Active non-pregnant; Open squares, Active Pregnant; Black triangles, Inactive non-pregnant; Open triangles, Inactive Pregnant. Small squares represent individual subjects; large squares represent group means, error bars are standard deviations. *p<0.01, †p<0.05.
pregnant and non-pregnant women (Non-pregnant: 4.4 ± 4.2%, Pregnant: 6.4 ± 3.2%, p=0.082). Fifty-five subjects per group would have been required to detect a significant difference with 80% power, given the means and standard deviations observed in the present sample. Fourth, L-FMC was not affected by physical activity. However, pregnancy and physical activity may alter the strength of the relationship between L-FMC and FMD.

**Artery Dependence of L-FMC:** Previous studies suggest that L-FMC occurs in the radial, but not the brachial, artery in healthy subjects. This study demonstrates that L-FMC is artery dependent in healthy pregnant and non-pregnant women. The L-FMC result contrasts with FMD, which occurs in the brachial, radial, axillary and superficial femoral arteries. The magnitudes of artery diameter changes during occlusion in the present study were similar to values reported previously in healthy subjects. Radial artery diameter decreased by 4.4 ± 4.2% in non-pregnant women, and 6.4 ± 3.2% in pregnant women. These values are comparable to previous studies reporting radial artery L-FMC of 5.2 ± 2% in healthy young subjects, 3.9 ± 1.1% in healthy young and middle aged subjects, and 6.8 ± 0.6% during saline infusion in healthy subjects. Brachial artery diameter did not change during forearm occlusion among non-pregnant women in the present study (0.5 ± 3.7%), however, small, but statistically significant, decreases were observed in pregnant women (-1.9 ± 3.2%, p=0.014). These values are similar to previous studies reporting changes in mean brachial artery diameter of -1.7%, -1.1%, and 2.4% during 5 minutes of forearm occlusion in healthy young men and women. In middle aged men and women, brachial artery diameter did not change during 5 minutes of forearm occlusion in healthy non-smokers, or during 5 minutes of wrist occlusion in normocholesterolemic control subjects. These six studies contrast with a single study reporting very large (≈15%) decreases in mean brachial artery diameter, despite a similar occlusion protocol and subject population. In
accordance with the results of the present study, the majority of studies observe no brachial artery
L-FMC in healthy subjects. Additional research is required to determine why L-FMC appears to
be artery dependant. Differences between the brachial and radial arteries in production, receptor
concentration, or smooth muscle responsiveness to endothelin-1, prostaglandins, and EDHF could
contribute to the artery-dependence of L-FMC.

The clinical significance of reduced L-FMC may also be artery dependent. Among healthy
subjects, L-FMC occurs in the radial,\(^1,7,9\) but not the brachial\(^{10-12,15}\) artery. The opposite results
are observed in clinical patients, in whom L-FMC occurs in the brachial artery,\(^{14,15,24}\) but is
attenuated in the radial artery.\(^1\) Radial artery L-FMC during 5 minutes of wrist occlusion was
significantly reduced in hypertensive patients compared to healthy controls, and by acute
smoking in patients with cardiovascular disease and hypertension.\(^1\) In contrast, brachial artery L-
FMC was observed during forearm occlusion in smokers,\(^14\) and during wrist occlusion in patients
with hypercholesterolemia,\(^15\) but not in healthy non-smoking\(^14\) or normocholesterolemic\(^15\) control
groups. Brachial artery vasoconstriction was correlated with total blood cholesterol when healthy
and hypercholesterolemic patients were pooled (\(r=0.72, p<0.0011\)).\(^15\) Three months of lipid
lowering therapy significantly reduced brachial artery L-FMC in hypercholesterolemic patients,
whereas three months of placebo administration had no effect.\(^24\) Further studies in diverse
populations are required to test the hypothesis that endothelial dysfunction is manifested as
enhanced brachial artery L-FMC, but attenuated radial artery L-FMC.

**Effect of Pregnancy on L-FMC:** Radial artery responsiveness to decreases in shear rate appear
to be unaffected by pregnancy. When women at 30-36 weeks gestation are compared to non-
pregnant subjects in the menstrual cycle phase most similar to pregnancy, radial artery L-FMC did not differ significantly between pregnant and non-pregnant women (Non-pregnant: 4.4 ± 4.2%, Pregnant: 6.4 ± 3.2%, p=0.082). Fifty-five subjects per group would have been required to detect a significant difference with 80% power, given the means and standard deviations observed in the present sample. Although L-FMC did not occur in the brachial artery, percent changes in brachial artery diameter during occlusion were significantly larger in pregnant (-1.9 ± 3.2%) than in non-pregnant (0.5 ± 3.7%) subjects. These results could be due to Type II error, or may indicate that elevated shear stress in pregnancy causes a minor dilation in the brachial artery at rest.

**Relationship Between L-FMC and FMD:** Two observations of previous studies suggested that L-FMC and FMD were independent measurements. First, a previous study observed no relationship between radial artery L-FMC and FMD in healthy or clinical subpopulations, or when all subjects were pooled.¹ In contrast to these observations, L-FMC and FMD were positively correlated in pregnant and non-pregnant women in the present study. The divergent results of the previous study¹ may have been due to several factors. First, the ability to detect a relationship in individual subpopulations may have been limited by sample size (13-20 subjects/group), as correlations did not reach statistical significance in most individual subgroups in that study.¹ Second, the relationship between L-FMC and FMD in clinical patients may differ from the relationship observed in healthy controls, resulting in no correlation when healthy and clinical patients were pooled in the previous study.¹ Third, radial artery L-FMC and FMD in the present study were based on an average of two trials, which may have reduced error.
The second observation in the literature which suggests that L-FMC and FMD are independent measurements is that single blockade of either endothelin-1 Type A receptors, prostaglandins, or EDHF significantly reduces L-FMC, but does not effect FMD. The interpretation of these observations is not clear, however, as these studies may not have included all factors that contribute to L-FMC. An unidentified factor that contributes to L-FMC could have delayed the time of return to pre-inflation diameter in control and experimental trials. Furthermore, single blockade does not account for interactions between factors regulating the occurrence and reversal of L-FMC, and FMD. Nitric oxide, prostaglandins, and EDHF interact to maintain resting radial artery diameter when the baseline shear stimulus is present. Radial artery diameter decreases significantly when all three vasodilators are simultaneously blocked by aspirin, fluconazole, and L-NMMA administration, however, does not change when these substances are administered separately, or during combined administration of aspirin and L-NMMA, or aspirin and fluconazole. If there are also interactions between factors regulating L-FMC and FMD after removal of the low-shear stimulus, this could obscure the relationship between L-FMC and FMD during single blockade studies.

The positive relationship between L-FMC and FMD in the present study is consistent with the hypothesis that FMD is a composite of hyperemia-induced dilation, superimposed on the normalization of factors regulating L-FMC following removal of the low-shear stimulus. The interpretation of L-FMC and FMD as independent measurements is based on the assumption that L-FMC has fully reversed before peak FMD occurs. This assumption should be tested. Studies should assess within-subject differences between the time course of peak FMD, and the time course of normalization of L-FMC following removal of the low-shear stimulus in the absence of reactive hyperemia. If the time at which peak FMD occurs is less than the time required for
diameter to return to pre-inflation values following cuff release when reactive hyperemia is prevented, then FMD may be influenced by the magnitude of L-FMC, and the time courses for peak FMD and the return to baseline levels of factors regulating L-FMC. This would have significant implications for the interpretation of reduced FMD when comparing subject groups which do, and do not, experience L-FMC. The factors regulating L-FMC, and the time required for each factor to normalize following the removal of the low-shear stimulus, should also be determined. Endothelin-1 contributes to L-FMC, as L-FMC is significantly attenuated by blockade of endothelin-1 Type A receptors with low doses of BQ-123. The role of prostaglandins, and EDHF, is less clear. Further study is required to explain the paradoxical finding that single blockade of the vasodilators EDHF and prostaglandin attenuates L-FMC. These results suggest a complex interaction between nitric oxide, prostaglandins, EDHF, endothelin-1, and shear stress, in regulating radial artery diameter prior to and during distal cuff inflation.

Effect of physical activity on the relationship between L-FMC and FMD: Compared to women of the same reproductive status, correlations between L-FMC and FMD were weaker among active than among inactive women. There was no relationship between L-FMC and FMD in active pregnant women in either the brachial or radial arteries. Additional studies with larger sample sizes are required to determine whether this is a real physiological effect, or reflects larger sample sizes in the active groups. Mechanistic studies described in the preceding section are also important to determine the clinical significance of this observation.
**Conclusions:** This is the first study using paired measurements to demonstrate that L-FMC is artery-dependant. L-FMC occurs in the radial artery of most healthy non-pregnant and pregnant women during 5 minutes of wrist occlusion, however, does not occur in the brachial artery during 5 minutes of forearm occlusion. Further studies are required to test the hypothesis that endothelial dysfunction is manifested as enhanced brachial artery L-FMC, but attenuated radial artery L-FMC. Radial artery L-FMC was not affected by pregnancy or physical activity. Positive correlations between L-FMC and FMD indicate that FMD is reduced among women who experience greater L-FMC. FMD may therefore be a composite of hyperemia-induced dilation, superimposed on the normalization of factors regulating L-FMC following removal of the low-shear stimulus. Additional studies are required to examine the mechanisms regulating L-FMC, and to determine whether normalization of the factors regulating L-FMC following removal of the low-shear stimulus occurs prior to peak FMD. The results of these studies may effect the interpretation of reduced FMD when comparing subject groups which do, and do not experience L-FMC.
5.6 References


Chapter 6
Discussion

This dissertation advances scientific knowledge concerning the effects of pregnancy and physical activity on processes involved in the pathophysiology of preeclampsia by making several novel contributions to the literature. Chapter 3 is the first study to examine the physiological mechanisms by which chronic exercise may affect preeclampsia risk, focusing on the effects of exercise on angiogenic markers which contribute to the development of preeclampsia. From this study, it was concluded that pregnant, non-smoking women who exercise regularly have lower levels of serum sFlt-1 and sFlt-1:PIGF, higher levels of serum PIGF, and are less likely to experience high serum sEng levels, than sedentary women. The beneficial effects of exercise on PIGF and sFlt-1:PIGF are most pronounced among active women exercising less than 270 minutes/week. This is also the first study to test the hypothesis that acute exercise during pregnancy may increase the risk of severe preeclampsia by exacerbating pathophysiological processes that lead to preeclampsia. The results demonstrate that anti-angiogenic changes that could contribute to preeclampsia did not occur immediately following short-duration, moderate-intensity exercise in the third trimester.

Chapter 4 is the first study to examine the effects of physical activity during pregnancy on conduit artery vascular function. This study also addresses limitations of previous studies examining the effects of pregnancy on flow-mediated dilation (FMD) by accounting for the shear stimulus, inter-individual variability in the timing of peak FMD, and menstrual cycle phase in non-pregnant controls. The results demonstrate that FMD and the shear stimulus for FMD are not
affected by pregnancy or regular exercise, although the relationship between FMD and the shear stimulus may be strengthened in active women.

Chapter 5 employs a newly proposed technique, low flow-mediated constriction (L-FMC), to assess conduit artery vascular responsiveness to decreased shear stress. In addition to examining the effects of pregnancy and regular exercise on L-FMC, this study reveals two important methodological findings. First, this is the first study to use paired brachial and radial artery measurements to demonstrate that L-FMC is artery dependent in healthy pregnant and non-pregnant women. Second, positive correlations between L-FMC and FMD question the interpretation that L-FMC and FMD are independent measurements.¹

Although the etiology of preeclampsia is incompletely defined, recent evidence suggests that excessive circulating soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) contribute to the pathophysiology of the syndrome.² The beneficial effects of regular exercise on angiogenic markers reported in Chapter 3 could contribute to the reduced risk of preeclampsia among active women,³ and the decreased risk of severe preeclampsia among women who are unexposed to smoke in pregnancy and exercise less than 270 minutes per week⁴ reported in prospective epidemiological studies. Data presented in this dissertation demonstrate that pregnant, non-smoking women who exercise regularly have lower levels of serum sFlt-1 and sFlt-1:PIGF, higher levels of serum PI GF, and are less likely to experience high serum sEng levels, than sedentary women. sFlt-1 and sEng were positively correlated with time spent watching television, and negatively correlated with indices of physical activity and exercise. Cross-sectional comparisons between pregnant and non-pregnant women suggest that among active women, proportionate pregnancy-induced increases in sFlt-1 and PI GF result in moderate increases in
sFlt-1:PlGF. In contrast, pregnancy-induced increases in sFlt-1 greatly exceed increases in PlGF among inactive women, and the increase in sFlt-1:PlGF is extremely large. Longitudinal studies with additional subjects are required to confirm these results, which suggest that increasing physical activity and exercise and decreasing time spent watching television may attenuate pregnancy-induced increases in sFlt-1, and prevent excessive increases in sEng. Regular exercise may also attenuate pregnancy-induced increases in sFlt-1. While sFlt-1 and sFlt-1:PlGF were significantly greater in sedentary women than in exercising women, the magnitude of differences between groups increased when women were subdivided by Active Living Index or Weighted Total Activity scores. Future studies should evaluate the independent effects of time spent on sedentary activities, physical activity, and exercise, on circulating levels of sFlt-1 and sEng, and preeclampsia risk.

Larger studies which measure angiogenic markers in normotensive active and inactive pregnant women, and women with preeclampsia are required to evaluate the clinical significance of the observed effects. The elevated sEng levels observed in three inactive women approach previously reported values from women with mild preeclampsia, but are lower than values reported in women with severe preeclampsia and HELLP syndrome (a variant of severe preeclampsia characterized by hemolysis, elevated liver enzymes, and low platelet counts). sFlt-1 in inactive pregnant women was also lower than previously reported values in mild and severe preeclampsia, and HELLP syndrome. These observations suggest that the anti-angiogenic effects of physical inactivity during pregnancy are not sufficient to cause preeclampsia, but may contribute to preeclampsia in women with other risk factors. The present study included healthy, non-smoking women with no history of hypertension. Further study is required to determine whether similar results would be observed in women with risk factors for preeclampsia, or in smokers.
The chronic effects of exercise on PI GF and sFlt-1:PI GF in the present study were most pronounced among active pregnant women exercising less than 270 minutes per week throughout pregnancy. These effects could contribute to the previously reported decrease in the risk of severe preeclampsia among women who were not exposed to smoke in pregnancy, and exercised less than 270 minutes/week during early pregnancy. These results do not explain the dramatic increase in risk among women exercising more than 270 minutes per week, compared to sedentary controls, as sFlt-1:PI GF and PI GF did not differ between these two groups. These preliminary results are based on small sample sizes (n=6-10/group), and should be confirmed in larger studies.

Furthermore, additional studies are needed to determine the actual weekly exercise duration at which the risk of severe preeclampsia increases. The study which observed an increase in the risk of severe preeclampsia among women exercising more than 270 minutes/week in early pregnancy constructed five exercise duration categories designed to include the activity durations most commonly reported by study participants. The categories below and above the 270 minutes/week threshold included all values between 150-269, and 270-419 minutes/week, respectively. These two categories span a 269 minute/week difference in activity levels; therefore it is unlikely that increases in the risk of preeclampsia occurred at 270 minutes/week. If it is assumed that the 270 minutes/week threshold is correct, women would need to exercise for 40 minutes/day to exceed this weekly threshold. Canadian guidelines recommend that pregnant women exercise 3-4 days/week, indicating that women would need to exercise for 90 minutes three days/week, or 69 minutes four days/week to exceed this threshold. The threshold at which weekly exercise exceeds what is required to obtain health benefits and maintain fitness during pregnancy, and
becomes exercise training designed to enhance athletic performance, has not been determined. Although Canadian guidelines recommend regular exercise to improve health and maintain fitness during pregnancy, training for athletic competition is not recommended. Additional research is required to assess the risks and benefits of high volume exercise training during pregnancy.

Researchers have also hypothesized that acute exercise may exacerbate the pathophysiological processes that lead to preeclampsia, contributing to the increased in the risk of severe preeclampsia among women exercising more than 270 minutes/week. In contrast to this hypothesis, anti-angiogenic changes that could contribute to preeclampsia were not observed following 20 minutes of acute, moderate-intensity exercise in the third trimester. With the exception of a significant increase in PlGF among active pregnant women, sFlt-1, PlGF, sFlt-1:PlGF and sEng did not change significantly following exercise in active or inactive pregnant women. There are three important limitations to this finding. First, angiogenic markers were only measured immediately post-exercise, and it is possible that changes could occur later during the recovery period. sFlt-1 increased significantly 30 minutes following maximal treadmill testing in 5 healthy men, however responses immediately post-exercise were not measured. Second, longer duration exercise may have had different effects. Although the 20 minutes of exercise participants completed during the present study is within the exercise guidelines for pregnant women, many active women in the present study exceeded this duration during their regular exercise sessions. The effects of longer duration exercise should therefore be examined. Third, higher intensity exercise may have had different effects. Increases in sFlt-1 30 minutes after maximal treadmill testing in 5 healthy men were strongly correlated with total peak VO2 consumption ($r^2=0.82$). sFlt-1 returned to resting levels within two hours of maximal treadmill testing, which is
consistent with reports that resting sFlt-1 did not differ between sedentary and exercise trained men (n=8/group). Baseline sFlt-1 tended to increase following two weeks of maximal exercise training in three sedentary men, however differences were not significant due to the number of subjects. Dramatic increases in exercise participation could therefore increase baseline sFlt-1, however, this effect should be confirmed in a larger sample, and may not apply to a moderate intensity exercise program, to trained subjects, or to pregnant women, who have higher sFlt-1 levels than non-pregnant women due to increased placental sFlt-1 production in pregnancy.

The second set of objectives for this dissertation focused on the effects of pregnancy and physical activity on conduit artery structure and function. Previous studies have hypothesized that increases in brachial artery diameter during pregnancy may be caused by shear-mediated vasodilation. In accordance with this hypothesis, baseline shear rate is elevated in pregnant women when compared to non-pregnant controls, and a slight vasoconstriction during occlusion was observed in pregnant women (-1.9 ± 3.2%), but not in non-pregnant controls (0.4 ± 3.6%). These results could be due to Type II error, or may indicate that elevated shear stress in pregnancy causes minor brachial artery dilation at rest. However, despite the small vasoconstriction during distal cuff inflation in pregnant women, inflation diameter was still 12% greater than pre-inflation diameter in non-pregnant controls (p<0.001). This increase in diameter is not due to decreased sympathetic tone, as muscle sympathetic nerve activity in normotensive women is increased in the third trimester when compared to post-partum measurements, and non-pregnant controls. Therefore, pregnancy-induced increases in brachial artery diameter are likely due to structural remodeling of the artery.
True peak FMD, and the shear stimulus for FMD, did not differ between non-pregnant controls in the mid to late luteal phase and pregnant women at 30-36 weeks gestation. The relationship between FMD and shear rate AUC was also preserved during pregnancy in active women, as regression lines were nearly identical in pregnant and non-pregnant subgroups. These results suggest that flow-mediated responsiveness to increased shear is preserved despite altered hemodynamic conditions in pregnancy. Previous studies reporting that FMD was significantly increased at 32\textsuperscript{15} and 35\textsuperscript{11} weeks gestation when compared to non-pregnant controls likely underestimated FMD in non-pregnant women, and overestimated the effect of pregnancy, due to two factors. First, neither study controlled for menstrual cycle phase.\textsuperscript{11, 15} FMD varies during the menstrual cycle in healthy young women, and the inclusion of women in the early follicular and early luteal phases\textsuperscript{16, 17} in previous studies\textsuperscript{11, 12, 15, 18-20} would have decreased percent FMD among non-pregnant women, increasing the magnitude of the difference between pregnant and non-pregnant women. Second, previous studies did not control for delayed peak dilation during pregnancy by using continuous measurements to identify true peak diameter. The 60s and 55-65s average methods underestimate percent FMD to a significantly greater extent in non-pregnant than in pregnant women (2.6-2.7\% vs. 1.6\%), as post-release diameter is measured near peak in pregnant women, but after diameter has declined in non-pregnant women. When combined with the inclusion of non-pregnant women in multiple phases, this underestimation bias may contribute to erroneous conclusions that FMD is increased in pregnancy. In accordance with this hypothesis, the study which used the 60s method reported the lowest FMD in non-pregnant women, and the largest difference between pregnant and non-pregnant women (Non-pregnant: 5.8 ± 2.1\%, 32 weeks: 11.2 ± 5.5\%).\textsuperscript{15} The significant pregnancy-induced increase in FMD observed by Dorup and colleagues (Non-pregnant: 7.2 ± 2.8\%, 35 weeks: 10.6 ± 4.4\%)\textsuperscript{11} is likely due to menstrual cycle phase, rather than underestimation bias. This study used the 45s method.\textsuperscript{11}
which reduced FMD by 1.8% and 2.1% in non-pregnant and pregnant women, respectively, in the present data set.

Regular exercise participation had no effect on FMD, or the shear stimulus for FMD, however, it may alter the strength of the relationship between FMD and the shear stimulus. FMD and shear rate AUC were positively correlated in active, but not inactive, pregnant and non-pregnant women. Larger studies are required to determine whether this reflects a real physiological effect, or was due to the smaller sample size in inactive groups. Studies should also determine whether this may explain why FMD and shear rate AUC are correlated in active young men, but not in studies which did not account for physical activity.

The observation that physical activity did not affect FMD in pregnant and non-pregnant women is consistent with other studies which observed no effect of regular exercise on brachial artery FMD in healthy men and women. Enhanced endothelial function may provide short-term compensation for exercise-induced increases in shear stress. Researchers have hypothesized that repeated shear stress exposure causes structural remodeling to reduce shear stress, and that endothelial function in healthy subjects returns to pre-training levels after structural remodeling is completed. This hypothesis requires verification. However, it is consistent with the observation that FMD does not differ between healthy, active and inactive men and women in most, but not all, studies. Data obtained in this dissertation extend these results to pregnant women, and address limitations of the only study examining the effect of physical activity on FMD in non-pregnant women by measuring FMD proximal to the occlusion cuff. While the majority of studies suggest that exercise training does not cause long-term increases in conduit artery endothelial function in healthy subjects, a recent study questions these results.
the brachial and superficial femoral arteries of national team swimmers and cyclists was increased when compared to sedentary controls. Furthermore, cyclists demonstrated greater increases in superficial femoral artery FMD than swimmers, whereas swimmers demonstrated greater increases in brachial artery FMD than cyclists. The contrasting results of this study may be due to two factors. First, there may be a dose-response relationship between exercise training and FMD. Brachial artery FMD may be increased in national team and highly trained athletes, but not in recreational athletes, or active members of the general population. The absence of an increase in brachial artery FMD among national team athletes in one study may have been because FMD was measured distal to the cuff. Second, previous studies measured brachial artery FMD, however tested athletes whose sports included predominantly lower-body exercise training (runners, skiers). Increases in FMD were greater in the trained limbs of swimmers and cyclists; therefore increases in FMD among active subjects may have been observed had researchers examined the superficial femoral artery.

While further study is required to clarify the relationship between regular exercise on FMD in healthy subjects, studies in clinical patients consistently show that exercise training causes long-term improvements in endothelial responses in patients with endothelial dysfunction resulting from a variety of conditions. Therefore, the observation that regular exercise participation did not increase brachial artery FMD in healthy pregnant and non-pregnant women does not invalidate the hypothesis that exercise may reduce preeclampsia risk by correcting endothelial dysfunction. Aerobic conditioning increases local endothelium-dependent dilation in patients with endothelial dysfunction resulting from aging and type 2 diabetes, while large muscle mass exercise improves systemic endothelial function in heart failure patients. If similar results are observed in women at risk for preeclampsia, training-induced correction of disease-related
endothelial dysfunction may prevent or attenuate the main pathological process leading to this disease.

L-FMC has recently been proposed as a companion vascular function test to FMD. Previous studies suggest that L-FMC occurs in the radial, but not the brachial, artery in healthy subjects. This is the first study to use paired brachial and radial artery measurements to confirm that L-FMC is artery dependant in healthy pregnant and non-pregnant women. This artery dependence contrasts with FMD, which occurs in the brachial, radial, axillary and superficial femoral arteries. Radial artery L-FMC was not affected by pregnancy or regular exercise, and mean radial artery L-FMC values of -4.4% in non-pregnant women and -6.4% in pregnant women are similar to previously reported mean values of -3.9 to -6.8% in healthy young and middle aged adults. Mean changes in brachial artery diameter during occlusion of 0.5% in non-pregnant women, and -1.9% in pregnant women, are also similar to previous studies which suggest that brachial artery L-FMC does not occur in healthy adults.

Three important issues must be resolved before L-FMC can be used as a clinical test. First, the mechanisms regulating L-FMC must be conclusively identified. Endothelin-1 contributes to L-FMC, as L-FMC is significantly attenuated by blockade of endothelin-1 Type A receptors with low doses of BQ-123. The role of prostaglandins, and endothelium derived hyperpolarizing factor (EDHF), is less clear. When the baseline shear stimulus is present, nitric oxide, prostaglandins, and EDHF interact to maintain resting radial artery diameter. Radial artery diameter decreases significantly when all three vasodilators are simultaneously blocked by aspirin, fluconazole, and L-NMMA administration, however, does not change when these
substances are administered separately,\textsuperscript{1} or during combined administration of aspirin and L-NMMA, or aspirin and fluconazole.\textsuperscript{44} Further study is required to explain the paradoxical finding that single blockade of the vasodilators EDHF and prostaglandin attenuates L-FMC. These results suggest a complex interaction between nitric oxide, prostaglandins, EDHF, endothelin-1, and shear stress, in controlling radial artery diameter prior to and during distal cuff inflation. Studies examining the mechanisms regulating L-FMC should also determine why L-FMC occurs in the radial, but not the brachial artery of healthy non-pregnant and pregnant women.

Second, researchers should test the hypothesis that endothelial dysfunction is manifested as enhanced brachial artery L-FMC, but reduced radial artery L-FMC. In healthy non-pregnant and pregnant women, L-FMC occurred in the radial, but not the brachial artery. However, four studies have reported opposite results in clinical populations.\textsuperscript{1,38,40,45} Radial artery L-FMC during 5 minutes of wrist occlusion was significantly reduced in hypertensive patients when compared to healthy controls, and by acute smoking in patients with cardiovascular disease and hypertension.\textsuperscript{1} In contrast, brachial artery L-FMC was observed during forearm occlusion in smokers,\textsuperscript{40} and during wrist occlusion in patients with hypercholesterolemia,\textsuperscript{38} but not in healthy non-smoking\textsuperscript{40} or normocholesterolemic\textsuperscript{38} control groups. Brachial artery vasoconstriction was correlated with total blood cholesterol when healthy and hypercholesterolemic patients were pooled ($r=0.72$, $p=0.001$).\textsuperscript{38} Three months of lipid lowering therapy significantly reduced brachial artery L-FMC in hypercholesterolemic patients, whereas three months of placebo administration had no effect.\textsuperscript{45} Additional studies which measure brachial and radial artery L-FMC in diverse groups of clinical patients, and healthy controls are needed. These studies should include comparisons of normotensive pregnant, and preeclamptic women.
Third, the relationship between L-FMC and FMD must be carefully evaluated. The positive relationship between L-FMC and FMD in the present study is consistent with the hypothesis that FMD is a composite of hyperemia-induced dilation, superimposed on the normalization of factors regulating L-FMC following removal of the low-shear stimulus. The interpretation of L-FMC and FMD as independent measurements is based on the assumption that L-FMC has fully reversed before peak FMD occurs. This assumption should be tested. Studies should assess within-subject differences between the time course of peak FMD, and the time course of normalization of L-FMC following removal of the low-shear stimulus in the absence of reactive hyperemia. If the time at which peak FMD occurs is less than the time required for diameter to return to pre-inflation values following cuff release when reactive hyperemia is prevented, then FMD may be influenced by the magnitudes of L-FMC and FMD, and the time courses for peak FMD and the normalization of factors regulating L-FMC. This would have significant implications for the interpretation of reduced FMD when comparing subjects which do, and do not, experience L-FMC. Once the factors regulating L-FMC are identified, studies should also compare the time of peak FMD with the time required for these factors to return to pre-inflation levels during reactive hyperemia.

The research completed for this dissertation demonstrates that pregnant, non-smoking women who exercise regularly have lower levels of serum sFlt-1 and sFlt-1:PIGF, higher levels of serum PIGF, and are less likely to experience high serum sEng levels, than sedentary women. The beneficial effects of exercise on PIGF and sFlt-1:PIGF are most pronounced among active women exercising less than 270 minutes/week, and may contribute to the reduced risk of preeclampsia among women who are unexposed to smoke in pregnancy, and exercise less than 270 minutes/week. Anti-angiogenic changes that could contribute to preeclampsia did not occur
immediately following short-duration, moderate-intensity exercise in the third trimester. Future studies should examine the effects of exercise on other processes that contribute to the pathophysiology of preeclampsia, including oxidative stress. The FMD and L-FMC studies indicate that vascular function not altered at 30-36 weeks gestation when compared to non-pregnant controls, and that regular exercise does not affect conduit artery responsiveness to increases or decreases in shear stress among apparently healthy non-smoking women. This is similar to results of previous studies, which indicate that brachial artery FMD is not increased in recreational athletes,24,26 or active members of the general population,30 compared to sedentary controls. However, studies in clinical patients consistently show that exercise training causes long-term improvements in endothelial responses in patients with endothelial dysfunction resulting from a variety of conditions.24,32-34 Aerobic conditioning increases local endothelium-dependent dilation in patients with endothelial dysfunction resulting from aging24,34 and type 2 diabetes,32 while large muscle mass exercise improves systemic endothelial function in heart failure patients.33 Endothelial dysfunction is a key feature of preeclampsia, and studies examining the effects of exercise on vascular function in women at risk for preeclampsia, or with endothelial dysfunction prior to or in early pregnancy, may enhance researchers understanding of the relationship between preeclampsia and exercise.
6.1 References


# Appendix A

## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Text</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ANP</td>
<td>Active non-pregnant</td>
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<tr>
<td>AP</td>
<td>Active pregnant</td>
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<tr>
<td>AT_1-AAs</td>
<td>Angiotensin receptor 1 autoantibodies</td>
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<td>AUC</td>
<td>Area under curve</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>DEE</td>
<td>Daily energy expenditure</td>
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<tr>
<td>EDHF</td>
<td>Endothelium-derived hyperpolarizing factor</td>
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<tr>
<td>Eng</td>
<td>Endoglin</td>
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<tr>
<td>FMD</td>
<td>Flow-mediated dilation</td>
</tr>
<tr>
<td>HELLP</td>
<td>Hemolysis, elevated liver enzymes, low platelets</td>
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<tr>
<td>HLA-C</td>
<td>Human leukocyte antigen C</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>INP</td>
<td>Inactive non-pregnant</td>
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<td>IP</td>
<td>Inactive pregnant</td>
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<tr>
<td>IU</td>
<td>International units</td>
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<tr>
<td>IUGR</td>
<td>Intrauterine growth restriction</td>
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<td>KIR</td>
<td>Killer immunoglobulin receptors</td>
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<tr>
<td>KPAS</td>
<td>Kaiser Physical Activity Survey</td>
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<td>L-FMC</td>
<td>Low flow-mediated constriction</td>
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<td>Abbreviation</td>
<td>Full Text</td>
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<tr>
<td>LTPA</td>
<td>Leisure time physical activity</td>
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<td>Max.</td>
<td>Maximum</td>
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<tr>
<td>MVPA</td>
<td>Mean voluntary physical activity</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PIGF</td>
<td>Placental growth factor</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
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<tr>
<td>s</td>
<td>Second</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>sEng</td>
<td>Soluble endoglin</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>Soluble fms-like tyrosine kinase-1</td>
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<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>uNK</td>
<td>Uterine natural killer</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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# Appendix B

## Recruitment Initiatives: February 2006 - February 2008

<table>
<thead>
<tr>
<th>Initiatives at Kingston’s Seven Largest Employers</th>
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<tbody>
<tr>
<td>Queen’s University</td>
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<tr>
<td>Posters placed around campus and in the Queen’s Gazette each month</td>
</tr>
<tr>
<td>Email announcement sent to all grad students every 4 months</td>
</tr>
<tr>
<td>Half page announcement sent to all female employees less than 41 yrs. of age (800 women - May 2007)</td>
</tr>
<tr>
<td>Emails sent to 550 female Queen’s employees under the age of 41; 100 flyers send to employees with no emails listed (January 2008)</td>
</tr>
<tr>
<td>Email announcement sent through RDSF Research Group; Queen’s Chinese Students Association listserv</td>
</tr>
<tr>
<td>Limestone District School Board</td>
</tr>
<tr>
<td>Announcements placed on First Class online message board every 2 months</td>
</tr>
<tr>
<td>Half page announcements placed in mailboxes of female employees under the age of 41 at 4 area secondary schools and 20 area primary schools</td>
</tr>
<tr>
<td>Kingston General Hospital</td>
</tr>
<tr>
<td>Half page announcement sent to all female employees less than 41 yrs. of age (1200 women - June 2007)</td>
</tr>
<tr>
<td>Announcements regularly placed in boards, FAPC5 waiting room, and Fetal Assessment Unit waiting room, KGH Spectrum (employee newsletter)</td>
</tr>
<tr>
<td>Announcement with a detachable form for contact information given to each woman prior to her 20 wk ultrasound appointment at the Fetal Assessment Unit (October 2007 – February 2008)</td>
</tr>
<tr>
<td>Hotel Dieu Hospital</td>
</tr>
<tr>
<td>Announcement placed in employee newsletter in November and December 2007</td>
</tr>
<tr>
<td>Announcements regularly placed in boards</td>
</tr>
<tr>
<td>CFB Kingston</td>
</tr>
<tr>
<td>Posters placed in fitness center, medical offices, children’s playgroup area, Canex store</td>
</tr>
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### Initiatives at Kingston’s Seven Largest Employers, continued

<table>
<thead>
<tr>
<th>Employer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>City of Kingston</td>
<td>Ad placed in employee newsletter and on the online employee message board</td>
</tr>
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### Other Initiatives

<table>
<thead>
<tr>
<th>Initiative</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empire Life Financial</td>
<td>Announcements placed in employee lunch room</td>
</tr>
<tr>
<td>Initiatives targeting Kingston lesbian community</td>
<td>Email announcement sent to Queen’s LGBTA (students), gay and lesbian Queen’s faculty and staff members, and Kingston Queer listserve</td>
</tr>
<tr>
<td>Networking through former participants</td>
<td>Email networking announcement sent to all program participants for the last 3 years; women were asked to forward the information on to friends, colleagues, and family members</td>
</tr>
<tr>
<td>Networking through friends and colleagues</td>
<td>Email networking announcement sent to friends and colleagues; each person was asked to forward the information on to friends, colleagues, and family members</td>
</tr>
<tr>
<td>Email networking through women who were not eligible to participate</td>
<td>Email networking announcement sent to all women who were not eligible; each woman was asked to forward the information on to friends, colleagues, and family members</td>
</tr>
<tr>
<td>Kingston Multisport, TriRudy, Kingston Road Runners Association</td>
<td>Announcement circulated multiple times through online message board or daily email for each organization</td>
</tr>
<tr>
<td>Kids Kingston</td>
<td>Multiple announcements placed on Pregnancy, Trying to Conceive, and General Health topics of forum</td>
</tr>
<tr>
<td>Baby, Kids, Family</td>
<td>Announcement placed on Facebook page</td>
</tr>
<tr>
<td>Poster Campaign</td>
<td>Posters placed at all area pools, gyms, fitness centers, running stores, yoga and prenatal yoga studios, maternity stores and malls, doctors offices, ultrasound clinics, after hours clinics</td>
</tr>
<tr>
<td>Community Midwives of Kingston</td>
<td>Announcement given to every woman in her information package at her first appointment (February 2006 – 2008)</td>
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<tr>
<td>Beautiful Beginning</td>
<td>Announcement given to every woman in her information package at registration</td>
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### Other Initiatives, continued

<table>
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<tr>
<th>Organization</th>
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<tr>
<td>Childbirth Kingston</td>
<td>Announcements placed in some information packets</td>
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<td>KFLNA Health Unit</td>
<td>Announcements placed in waiting area</td>
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<td>North Kingston Community Health Center</td>
<td>Announcements placed in waiting area</td>
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<td>Kingston this Week</td>
<td>Announcements placed in the Community Digest</td>
</tr>
<tr>
<td>Heart &amp; Stroke Foundation</td>
<td>Attended Kingston Fall Leisure Fair at Heart &amp; Stroke booth to distribute recruitment information (September 2007)</td>
</tr>
</tbody>
</table>

### Organizations that were not able to help or did not respond

- Providence Care
- StarTek
- Brown’s Fine Foods
- Queen’s University Staff Association
- RMC
Appendix C

Physical Activity Readiness Screening Forms

Screening forms used in this study can be downloaded free of change from the Canadian Society for Exercise Physiology Website, at: http://www.csep.ca/english/view.asp?x=698. The PAR-Q was used to screen non-pregnant women, whereas pregnant women completed the PARmed-X for Pregnancy with their doctor, obstetrician, or midwife (see Pre-test Screening subsection of Methods section for Chapters 3, 4, and 5).
## PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly. Check YES or NO.

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?</td>
<td></td>
</tr>
<tr>
<td>2. Do you feel pain in your chest when you do physical activity?</td>
<td></td>
</tr>
<tr>
<td>3. In the past month, have you had chest pain when you were not doing physical activity?</td>
<td></td>
</tr>
<tr>
<td>4. Do you lose your balance because of dizziness or do you ever lose consciousness?</td>
<td></td>
</tr>
<tr>
<td>5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?</td>
<td></td>
</tr>
<tr>
<td>6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?</td>
<td></td>
</tr>
<tr>
<td>7. Do you know of any other reason why you should not do physical activity?</td>
<td></td>
</tr>
</tbody>
</table>

### If you answered NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- **start becoming much more physically active** — begin slowly and build up gradually. This is the safest and easiest way to go.
- **take part in a fitness appraisal** — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live activity. It is also highly recommended that you have your blood pressure measured. If your resting heart rate is over 144/94, talk with your doctor before you start becoming much more physically active.

### DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a flu — wait until you feel better.
- If you are or may be pregnant — talk to your doctor before you start becoming more active.

### PLEASE NOTE:

If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional.

Ask whether you should change your physical activity plan.

### Informed Use of the PAR-Q

The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity and/or in doubt after completing this questionnaire, consult your doctor prior to physical activity.

### No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

**Note:** If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

<table>
<thead>
<tr>
<th>NAME</th>
<th>DATE</th>
</tr>
</thead>
</table>

**Signature of participant:**

**Informed consent** (for participants under the age of 16 years):

**Note:** This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

continued on other side...
Physical Activity Readiness Questionnaire: PAR-Q+ (revised 2002)

PAR-Q & YOU

Choose a variety of activities from these three groups:

1. Increase physical activity
2. Improve physical fitness
3. Lose body fat

Getting Your Way, Every Day – For Life

Get Active! Physical activity is key to healthy living. By adding physical activity to your daily life, you can improve your health and fitness. Here are some tips to help you get started:

1. Start small:
   - Try a new activity every day
   - Start with 10-15 minutes
   - Increase gradually

2. Make it fun:
   - Choose activities you enjoy
   - Find a partner or group

3. Stay motivated:
   - Set achievable goals
   - Reward yourself

You Can Do It – Getting started is easier than you think!

Physical activity doesn’t have to be too hard. Start small and build up gradually. Here are some tips to help you get started:

1. Start with 10-15 minutes:
   - Increase gradually
   - Find a partner or group

2. Make it fun:
   - Choose activities you enjoy
   - Find a partner or group

3. Stay motivated:
   - Set achievable goals
   - Reward yourself

Benefits of regular activity:

- Better health and fitness
- Improved mood and energy
- Better sleep
- Weight management
- Reduced risk of chronic diseases


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FITNESS AND HEALTH PROFESSIONALS MAY BE INTERESTED IN THE INFORMATION BELOW:

The following companion forms are available for doctors’ use by contacting the Canadian Society for Exercise Physiology (address below):

1. Physical Activity Readiness Medical Examination (PARmed-X) – to be used by doctors with people who answer YES to one or more questions on the PAR-Q.

2. Physical Activity Readiness Medical Examination for Pregnancy (PARmed-X for Pregnancy) – to be used by doctors with pregnant patients who wish to become more active.

References:


For more information, please contact:

Canadian Society for Exercise Physiology
202-135 Somerset Street West
Ottawa, ON, K2P 0G2
Tel. 1-877-651-3765 • FAX (613) 234-3565
Online: www.csep.ca

The original PAR-Q was developed by the British Columbia Ministry of Health. It has been revised by an expert advisory committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Goldthwait (2002).

PARmed-X for PREGNANCY

PARmed-X for PREGNANCY is a guideline for health screening prior to participation in a prenatal fitness class or other exercise.

Healthy women with uncomplicated pregnancies can integrate physical activity into their daily living and can participate without significant risks either to themselves or to their unborn child. Potential benefits of such programs include improved aerobics and muscular fitness, promotion of appropriate weight gain, and facilitation of labour. Regular exercise may also help to prevent gestational glucose intolerance and pregnancy-induced hypertension.

The safety of prenatal exercise programs depends on an adequate level of maternal-fetal physiological reserve. PARmed-X for PREGNANCY is a convenient checklist and prescription for use by health care providers to evaluate pregnant patients who want to enter a prenatal fitness program and for ongoing medical surveillance of exercising pregnant patients.

Instructions for use of the 4-page PARmed-X for PREGNANCY are the following:

1. The patient should fill out the section on PATIENT INFORMATION and the PRE-EXERCISE HEALTH CHECKLIST (PART 1, 2, and 4 on p. 1) and give the form to the health care provider managing her pregnancy.
2. The health care provider should check the information provided by the patient for accuracy and fill out SECTION C on CONTRAINDICATIONS (p. 2) based on current medical information.
3. If no exercise contraindications exist, the HEALTH EVALUATION FORM (p. 3) should be completed, signed by the health care provider, and given to the patient to her prenatal fitness professional.

In addition to prudent medical care, participation in appropriate types, intensities, and amounts of exercise is recommended to increase the likelihood of beneficial pregnancy outcome. PARmed-X for PREGNANCY provides recommendations for individualized exercise prescription (p. 3) and program safety (p. 4).

NOTE: Sections A and B should be completed by the patient before the appointment with the health care provider.

### A PATIENT INFORMATION

- **NAME:**
- **ADDRESS:**
- **TELEPHONE:**
- **BIRTHDATE:**
- **HEALTH INSURANCE NO:**
- **PRESENTED FITNESS LEVEL:**
- **PROFESSIONAL’S PHONE NUMBER:**

### B PRE-EXERCISE HEALTH CHECKLIST

**PART 1: GENERAL HEALTH STATUS**

In the past, have you experienced (YES or NO):

1. Miscarriage in an earlier pregnancy? □ □
2. Other pregnancy complications? □ □
3. I have completed a PPA-Q within the last 30 days.
   - If answered YES to question 1 or 2, please explain:

   Number of previous pregnancies: ______

**PART 2: STATUS OF CURRENT PREGNANCY**

**Due Date:**

During this pregnancy, have you experienced:

1. Marked fatigue? □ □
2. Bleeding from the vagina ("spotting")? □ □
3. Unexplained absence of nausea? □ □
4. Unexplained abdominal pain? □ □
5. Sudden swelling of ankles, hands or face? □ □
6. Persistent headaches or problems with headaches? □ □
7. Swelling, pain or edema in the calf of one leg? □ □
8. Absence of fetal movement after 18th month? □ □
9. Failure to gain weight after 12th month? □ □
10. If answered YES to any of the above questions, please explain.

### PART 3: ACTIVITY HABITS DURING THE PAST MONTH

1. List only regular fitness/recreational activities:

<table>
<thead>
<tr>
<th>INTENSITY</th>
<th>FREQUENCY</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy</td>
<td>1-2</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Medium</td>
<td>2-4</td>
<td>20-40</td>
</tr>
<tr>
<td>Light</td>
<td>4+</td>
<td>40+</td>
</tr>
</tbody>
</table>

2. Does your regular occupation (job/home) activity involve:

   - Heavy lifting? □ □
   - Frequent walking/step climbing? □ □
   - Occasional walking (<10 steps)? □ □
   - Prolonged standing? □ □
   - Mainly sitting? □ □
   - Normal daily activity? □ □
   - Do you currently smoke tobacco? □ □
   - Do you consume alcohol? □ □

### PART 4: PHYSICAL ACTIVITY INTENTIONS

What physical activity do you intend to do?

- Is this a change from what you currently do? □ □

### NOTE: PREGNANT WOMEN ARE STRONGLY ADVISED NOT TO SMOKING OR CONSUME ALCOHOL DURING PREGNANCY AND DURING LACTATION.
CONTRAINDICATIONS TO EXERCISE: to be completed by your health care provider

<table>
<thead>
<tr>
<th>Absolute Contraindications</th>
<th>Relative Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does the patient have:</td>
<td>Does the patient have:</td>
</tr>
<tr>
<td>1. Prolapsed membranes, premature labour?</td>
<td>YES NO</td>
</tr>
<tr>
<td>2. Persistent second or third trimester bleeding; placenta previa?</td>
<td>YES NO</td>
</tr>
<tr>
<td>3. Pregnancy-induced hypertension or pre-eclampsia?</td>
<td>YES NO</td>
</tr>
<tr>
<td>4. Incompliant uterus?</td>
<td>YES NO</td>
</tr>
<tr>
<td>5. Evidence of intrauterine-growth restriction?</td>
<td>YES NO</td>
</tr>
<tr>
<td>6. High-order pregnancy (e.g., triplets)?</td>
<td>YES NO</td>
</tr>
<tr>
<td>7. Uncontrolled Type I diabetes, hypertension or thyroid disease, other serious cardiovascular, respiratory or systemic disorder?</td>
<td>YES NO</td>
</tr>
</tbody>
</table>

PHYSICAL ACTIVITY RECOMMENDATION: q Recommended/Approved q Contraindicated

Prescription for Aerobic Activity

RATE OF PROGRESSION: The best time to progress is during the second trimester since risks and discomforts of pregnancy are lowest at that time. Aerobic exercise should be increased gradually during the second trimester from a minimum of 10 minutes per session, 3 times per week (the approximate target heart rate is 150 BPM) to an amount of approximately 30 minutes per session, 4 times per week (at the approximate target heart rate or RPE).

WARM-UP/COLD-DOWN: Aerobic activity should be preceded by a brief (10-15 min) warm-up and followed by a short (10-15 min) cool-down. Low intensity calisthenics, stretching and relaxation exercises should be included in the warm-up/cold-down.

FREQUENCY: Exercise 3 times per week and progress to 4 times per week.

INTENSITY: Exercise within an appropriate RPE range and/or target heart rate zone.

TIME: At least 15 minutes, even if it means reducing the intensity (rest intervals may be helpful).

TYPE: High-intensity interval training or moderate endurance exercise using large muscle groups (e.g., walking, stationary cycling, swimming, aquatic exercise, or interval training).

"TALK TEST": An ideal check to avoid overexertion. Use the "talk test." The exercise intensity is excessive if you cannot carry on a verbal conversation while exercising.

The original PARmed-X for PREGNANCY was developed by L.A. Watts, Ph.D., Queen's University. The muscular conditioning component was developed by M.F. Metcalf, Ph.D., University of Western Ontario. The document has been revised based on advice from an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Girdell with additional input from Drs. Waddell and Motola, and Gregory A.L. Davies, M.D., FRCPC (Department of Obstetrics and Gynecology, Queen's University, 2003).

No changes permitted. Translation and reproduction in its entire is encouraged.

Additional copies of the PARmed-X for PREGNANCY, the PARmed-X and/or the PAR can be downloaded from: http://www.csep.ca/parex.pdf

For more information contact the Canadian Society for Exercise Physiology

18G Grenville St. Suite 600, Ottawa, Ontario CANADA K1P 6J9
Tel: 1 613 524 3055 Fax: 1 613 524 3055 www.csep.ca

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PARmed-X for PREGNANCY

PHYSICAL ACTIVITY READINESS
MEDICAL EXAMINATION

Prescription for Muscular Conditioning

It is important to condition all major muscle groups during both prenatal and postnatal periods.

**EXAMPLES OF MUSCULAR STRENGTHENING EXERCISES**

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>PURPOSE</th>
<th>EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper back</td>
<td>Promotion of good posture</td>
<td>Shoulder shrugs, shoulder blade pinch</td>
</tr>
<tr>
<td>Lower back</td>
<td>Promotion of good posture</td>
<td>Modified standing opposite leg &amp; arm lifts</td>
</tr>
<tr>
<td>Abdomen</td>
<td>Promotion of good posture, prevent low back pain, prevent diastasis recti, strengthen muscles of labour</td>
<td>Abdominal tightening, abdominal curl-ups, head raises lying on side or standing position</td>
</tr>
<tr>
<td>Pelvic floor</td>
<td>Promotion of good bladder control, prevention of urinary incontinence, &quot;Wave&quot;, &quot;Elevator&quot;</td>
<td></td>
</tr>
<tr>
<td>Upper body</td>
<td>Improve muscular support for breath</td>
<td>Shoulder rotations, modified push-ups against a wall</td>
</tr>
<tr>
<td>Buttocks</td>
<td>Facilitation of weight bearing, prevention of varicose veins</td>
<td>Barbel squats, standing leg lifts, head raises</td>
</tr>
</tbody>
</table>

**PRECAUTIONS FOR MUSCULAR CONDITIONING DURING PREGNANCY**

**VARIABLE** | **EFFECTS OF PREGNANCY** | **EXERCISE MODIFICATIONS** |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Position</td>
<td>In the supine position lying on the back, the enlarged uterus may either decrease the flow of blood returning from the lower half of the body, or it may decrease flow to a major artery (abdominal aorta)</td>
<td>Past 4 months of gestation; exercises normally done in the supine position should be avoided; each exercise should be done lying on the side</td>
</tr>
<tr>
<td>Joint Laxity</td>
<td>Ligaments become relaxed due to increasing hormone levels</td>
<td>Avoid rapid changes in direction and duration during exercises; stretching should be performed with controlled movements</td>
</tr>
<tr>
<td>Abdominal Muscles</td>
<td>Presence of a slipping (hanging) of connective tissue along the midline of the pregnant abdomen (diastasis recti) may be seen during abdominal exercises</td>
<td>Abdominal exercises are not recommended if diastasis recti develops</td>
</tr>
<tr>
<td>Posture</td>
<td>Increasing weight of enlarged breasts and uterus may cause a forward shift in the Centre of Gravity and may increase the arch in the lower back. This may also cause shoulders to hump forward</td>
<td>Emphasis on correct posture and neutral pelvic alignment; Neutral pelvic alignment is found by bending the knees, feet shoulder width apart, and aligning the spine between accentuated lordosis and the posterior sacrum in position</td>
</tr>
</tbody>
</table>

**Precautions for Resistance Exercises** | **EFFECTS OF PREGNANCY** |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Emphasis must be placed on continuous breathing throughout exercise</td>
<td></td>
</tr>
<tr>
<td>Inhale on exertion, exhale on relaxation using high repetitions and low weights</td>
<td></td>
</tr>
<tr>
<td>Valsava Maneuver (holding breath while working against a resistance) causes a change in blood pressure and therefore should be avoided</td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**PARmed-X for Pregnancy - Health Evaluation Form**

(to be completed by patient and given to the prenatal fitness professional after obtaining medical clearance to exercise)

1. PLEASE PRINT (patient’s name), have discussed my plans to participate in physical activity during my current pregnancy with my health care provider and I have obtained his/her approval to begin participation.

Signed: ________________________ Date: ________________________

(patient’s signature)

Name of health care provider: ________________________

Address: ________________________

______________________________

______________________________

______________________________

______________________________

Telephone: ________________________

______________________________

(health care provider’s signature)

HEALTH CARE PROVIDER’S COMMENTS:

______________________________

______________________________

______________________________

______________________________

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Advice for Active Living During Pregnancy

Pregnancy is a time when women can make beneficial changes in their health habits to protect and promote the healthy development of their unborn babies. These changes include adopting improved eating habits, abstaining from smoking and alcohol intake, and participating in regular moderate physical activity. Since all of these changes can be carried over into the postnatal period and beyond, pregnancy is a very good time to adopt healthy lifestyle habits that are permanent by integrating physical activity with enjoyable healthy eating and a positive self and body image.

**Active Living:**
- see your doctor before increasing your activity level during pregnancy
- exercise regularly but don't overdo it
- exercise with a pregnant friend or join a prenatal exercise program
- follow FITT principles modified for pregnant women
- know safety considerations for exercise in pregnancy

**Healthy Eating:**
- the need for calories is higher (about 400 more per day) than before pregnancy
- follow Canada's Food Guide to Healthy Eating and choose healthy foods from the following groups: whole grain or enriched bread or cereal; fruits and vegetables; milk and milk products; meat, fish, poultry and alternatives
- drink 6-8 glasses of fluid, including water, each day
- salt intake should not be restricted
- limit caffeine intake i.e., coffee, tea, chocolate, and cola drinks
- dieting to lose weight is not recommended during pregnancy

**Positive Self and Body Image:**
- remember that it is normal to gain weight during pregnancy
- accept that your body shape will change during pregnancy
- enjoy your pregnancy as a unique and meaningful experience

---

**SAFETY CONSIDERATIONS**
- Avoid exercise in warm/humid environments, especially during the 1st trimester
- Avoid isometric exercise or straining while holding your breath
- Maintain adequate nutrition and hydration — drink liquids before and after exercise
- Avoid exercise while lying on your back past the 4th month of pregnancy
- Avoid activities which involve physical contact or danger of falling
- Know your limits — pregnancy is not a good time to train for athletic competition
- Know the reasons to stop exercise and consult a qualified health care provider immediately if they occur

**REASONS TO STOP EXERCISE AND CONSULT YOUR HEALTH CARE PROVIDER**
- Excessive shortness of breath
- Chest pain
- Painful uterine contractions (more than 6-8 per hour)
- Vaginal bleeding
- Any "gush" of fluid from vagina (suggesting premature rupture of the membranes)
- Dizziness or faintness
Appendix D

Three Day Physical Activity Record

Subjects completed a 3-day physical activity record on consecutive days (one weekend day, two weekdays) within 2 weeks of the test to evaluate current physical activity (see Physical Activity Indices subsection of the Methods section of Chapters 3, 4, and 5). Additional information concerning validation and use of the 3-day physical activity record is presented in:


Note: Only one daily physical activity record sheet is shown, however, subjects were given three daily record sheets (one for each day).
3-DAY PHYSICAL ACTIVITY RECORD

1. Complete the 3-DAY PHYSICAL ACTIVITY RECORD for 3 days (preferably consecutive days), including one weekend day and two weekdays (i.e. Thursday, Friday, Saturday or Sunday, Monday, Tuesday). Please try to select days that are representative of your usual activity levels (i.e. avoid days where you are sick, traveling, participating in an athletic competition, etc.). Record each day on a separate activity form. This PHYSICAL ACTIVITY RECORD will allow us to examine the effects of your level of physical activity on physiological variables associated with the development of preeclampsia.

2. Each 24-hour day is divided into 96 periods of 15 minutes. For each 15 minute period, enter a categorical value (see attached TABLE) ranging from 1 to 9, corresponding to the dominant activity for the period (i.e. Sleeping → value of 1).

Example: Zero hour is midnight; hour 1 is 1am, and so on. The most usual activity for this time of day is sleeping. Therefore, for the zero hour, the categorical value of 1 is recorded in each of the four 15 minute blocks. Repeat the latter step for the rest of the day.

<table>
<thead>
<tr>
<th>Minute →</th>
<th>0-15</th>
<th>16-30</th>
<th>31-45</th>
<th>46-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour ↓</td>
<td></td>
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<tr>
<td>0</td>
<td></td>
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<tr>
<td>2</td>
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</tbody>
</table>

Table of Activities & Corresponding Categorical Values

<table>
<thead>
<tr>
<th>Value</th>
<th>Description: Examples of Activities in Each Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sleeping, resting in bed</td>
</tr>
<tr>
<td>2</td>
<td>Sitting, eating, listening, writing, etc.</td>
</tr>
<tr>
<td>3</td>
<td>Light activity: standing, washing shaving, combing, cooking, etc.</td>
</tr>
<tr>
<td>4</td>
<td>Slow walking (&lt; 4 km/h / 2.5 mph), driving, dressing, showering, etc.</td>
</tr>
<tr>
<td>5</td>
<td>Light manual work: floor sweeping, window washing, driving a truck, painting, waiting on tables, nursing chores, several hours chores, electrician, barman, waling at 4-6 km/h (2.5-4.7 mph), etc.</td>
</tr>
<tr>
<td>6</td>
<td>Leisure activities and sports in a recreational environment: baseball, golf, volleyball, canoeing or rowing, archery, bowling, cycling&lt;10 km/h (6.2 mph), table tennis, etc.</td>
</tr>
<tr>
<td>7</td>
<td>Manual work at a moderate pace: mining, carpentry, house building, lumbering, wood cutting, show shoveling, loading and unloading goods, etc.</td>
</tr>
<tr>
<td>8</td>
<td>Leisure sports and activities of higher intensity (not competitive): canoeing (5-8 km/h or 3-5 mph), bicycling &gt; 15 km/h (9 mph), dancing, skiing, badminton, gymnastics, swimming, tennis, horseback riding, walking &gt; 6 km/h (3.7 mph), etc.</td>
</tr>
<tr>
<td>9</td>
<td>Intense manual work, high intensity sport activities or sport competition: tree cutting, carrying heavy loads, jogging and running &gt; 9 km/h (5.6 mph), racquetball, badminton, swimming, tennis, cross-country skiing &gt; 8 km/h (4.8 mph), hiking and mountain climbing, etc.</td>
</tr>
</tbody>
</table>
EXERCISE & PREGNANCY STUDY THREE-DAY PHYSICAL ACTIVITY RECORD

Please complete during the week of:  Subject #:

Date:  First day of last menstrual period:

Day:  M  T  W  Th  F  Sa  Su

In each square, record the Categorical Value (1 to 9) that BEST corresponds to the DOMINANT activity of each 15 min period. Consult the activity card to establish the proper value. If you’re unsure, make a note and discuss your concern with the study coordinator.

<table>
<thead>
<tr>
<th>Minute →</th>
<th>0-15</th>
<th>16-30</th>
<th>31-45</th>
<th>46-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour ↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
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<tr>
<td>1</td>
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Appendix E
Kaiser Physical Activity Survey

Chronic physical activity was evaluated using the Kaiser Physical Activity Survey (KPAS), as described in the Physical Activity Indices subsection of the Methods section of Chapters 3, 4, and 5. Additional information concerning the validation of the KPAS in non-pregnant and pregnant women, use of the KPAS, and calculation of physical activity indices, is contained in:


**Kaiser Physical Activity Survey: Non-Pregnant**

**Section 1: Household & Family Care Activities**

First we want to know about your activities at home, not including activities you may do at your home or other people’s home for pay. During the past 12 months, how much time did you spend:

<table>
<thead>
<tr>
<th>Activity Description</th>
<th>Time Spent Options</th>
<th>Score Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Caring for a child or children under 2 years of age?</td>
<td>None or &lt; 1 hr / week / ≥ 1 hr but &lt; 20 hrs / week / ≥ 20 hours / week</td>
<td>1 – 3 – 5</td>
</tr>
<tr>
<td>2. Caring for children between 2 and 5 years of age?</td>
<td>None or &lt; 1 hr / week / ≥ 1 hr but &lt; 20 hrs / week / ≥ 20 hours / week</td>
<td>1 – 3 – 5</td>
</tr>
<tr>
<td>3. Caring for a disabled child or elderly person? (only count time spent actually</td>
<td>None or &lt; 1 hr / week / ≥ 1 hr but &lt; 20 hrs / week / ≥ 20 hours / week</td>
<td>1 – 3 – 5</td>
</tr>
<tr>
<td>4. Preparing meals or cleaning up from meals on weekdays?</td>
<td>None or &lt; ½ hr/day / ≥ ½ hr but &lt; 1 hr/day / ≥ 1 hr but &lt; 1½ hr/day / ≥ 1½ hr but &lt; 2 hr/day / ≥2 hr/day</td>
<td>1 – 2 - 3 – 4 - 5</td>
</tr>
<tr>
<td>5. Preparing meals or cleaning up from meals on weekends?</td>
<td>None or &lt; ½ hr/day / ≥ ½ hr but &lt; 1 hr/day / ≥ 1 hr but &lt; 1½ hr/day / ≥ 1½ hr but &lt; 2 hr/day / ≥2 hr/day</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>6. Doing major cleaning such as shampooing carpets, waxing floors, or washing</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week /</td>
<td>1 – 2 - 3 – 4 – 5</td>
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<tr>
<td>7. Doing routine cleaning such as dusting, laundry, vacuuming, or changing linens?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week /</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>8. Going grocery shopping or pushing a shopping cart?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week /</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>9. Doing gardening or yard work, such as mowing the lawn or raking leaves?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week /</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
</tbody>
</table>
10. Doing heavy outdoor work, such as chopping wood, tilling soil, shoveling snow, or baling hay?
   Never or less than once a month / Once a month / 2-3 times a month / Once a week / More than once a week

11. Doing major home decoration or repair, such as plumbing, tiling, painting or building?
   Never or less than once a month / Once a month / 2-3 times a month / Once a week / More than once a week

SECTION II: OCCUPATIONAL ACTIVITIES

Now, some questions about your employment situation during the past year.

12. What is your occupation (if more than one job, describe your occupation for the job with the most hours worked per week)?

13. What is the name of your employer, business or company?

14. What kind of business or industry is this? (For example, hospital, university, newspaper publishing, mail order house, auto engine manufacturing, etc.)

15. What are your most important specific activities? (For example, selling cars, keeping account books, etc.)
   a.
   b.
   c.

16. Which best describes your current occupation:
   a. Employee of a private company, business or individual for wages, salary, or commissions
   b. Employee of Federal government
   c. Employee of a state or local government
   d. Self-employed in own business, professional practice or farm
   e. Working without pay in a home, family business or farm
17. In comparison with other women your age, do you think your work is **physically**…
   Much lighter / Lighter / The same as / Heavier / Much heavier

18. After work, are you **physically** tired?
   Never / Seldom / Sometimes / Often / Always

19. When you are working at your current occupation, how often do you do each of the following?

<table>
<thead>
<tr>
<th></th>
<th>Never / Seldom / Sometimes / Often / Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Sit:</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>b. Stand:</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>c. Walk:</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>d. Lift heavy loads:</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>e. Sweat from exertion:</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
</tbody>
</table>
### SECTION III: ACTIVE LIVING HABITS

This next section asks about the general level of physical activity involved in your daily routine during the past year.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
<th>Options</th>
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</thead>
<tbody>
<tr>
<td>20. How many minutes a day do you usually walk and/or bicycle to and from work, school or errands?</td>
<td>&lt; 5 / ≥ 5 but &lt; 15 / ≥ 15 but &lt; 30 / ≥ 30 but &lt; 45 / ≥ 45</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>21. Did you watch television?</td>
<td>&lt; 1 hour/week / ≥ 1 hour/week but &lt; 1 hour/day / ≥ 1 hour/day but &lt; 2 hours/day / ≥ 2 hours/day but &lt; 4 hours/day / ≥ 4 hours a day</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>22. Did you walk (for at least 15 minutes at a time)?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week / More than once a week</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>23. Did you bike (for at least 15 minutes at a time)?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week / More than once a week</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
</tbody>
</table>

### SECTION IV: PARTICIPATION IN SPORTS AND EXERCISE

Finally, we want to ask about your participation in exercise during the past year.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
<th>Options</th>
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<tbody>
<tr>
<td>24. In comparison with other women of your own age, do you think your recreational physical activity is…</td>
<td>Much less / Less / The same as / More / Much More</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>25. Did you play sports or exercise?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week / More than once a week</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>26. Did you sweat from exertion during sports or exercise?</td>
<td>Never or less then once a month / Once a month / 2-3 times a month / Once a week / More than once a week</td>
<td>1 – 2 - 3 – 4 – 5</td>
</tr>
<tr>
<td>27. During the past year, did you participate in any of these activities or in any other similar activities not included in the list?</td>
<td>Yes / No</td>
<td></td>
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<tr>
<td>28. Which sport or exercise did you do most frequently?</td>
<td></td>
<td>Intensity: 0.76 – 1.26-1.76</td>
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<tr>
<td>Question</td>
<td>Options/Proportion/Time</td>
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<tr>
<td>29. How many months in the past year did you do this activity?</td>
<td>&lt;1 / 1-3 / 4-6 / 7-9 / &gt;9</td>
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<td></td>
<td>Proportion: 0.5 - 1.3 - 2.5 - 3.5 - 4.5</td>
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<tr>
<td>30. How many hours per week did you usually do this activity?</td>
<td>&lt;1 / ≥1 but &lt; 2 / ≥2 but &lt; 3 / ≥3 but &lt; 4 / ≥4</td>
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<td></td>
<td>Time: 0.04 - 0.17 - 0.42 - 0.67 - 0.92</td>
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<tr>
<td>31. What was your typical rating of perceived exertion while doing this activity?</td>
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<tr>
<td>32. Did you do any other exercise or play any other sport this past year?</td>
<td>Yes / No</td>
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<td>33. What was the second most frequent sport or exercise you did?</td>
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<td></td>
<td>Intensity: 0.76 – 1.26-1.76</td>
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<tr>
<td>34. How many months in the past year did you do this activity?</td>
<td>&lt;1 / 1-3 / 4-6 / 7-9 / &gt;9</td>
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<td></td>
<td>Proportion: 0.5 - 1.3 - 2.5 - 3.5 - 4.5</td>
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<tr>
<td>35. How many hours per week did you usually do this activity?</td>
<td>&lt;1 / ≥1 but &lt; 2 / ≥2 but &lt; 3 / ≥3 but &lt; 4 / ≥4</td>
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<td></td>
<td>Time: 0.04 - 0.17 - 0.42 - 0.67 - 0.92</td>
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<tr>
<td>36. What was your typical rating of perceived exertion while doing this activity?</td>
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<tr>
<td>37. Did you do any other exercise or play any other sport this past year?</td>
<td>Yes / No</td>
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<td>38. What was the third most frequent sport or exercise you did?</td>
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<td></td>
<td>Intensity: 0.76 – 1.26-1.76</td>
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<tr>
<td>39. How many months in the past year did you do this activity?</td>
<td>&lt;1 / 1-3 / 4-6 / 7-9 / &gt;9</td>
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<td></td>
<td>Proportion: 0.5 - 1.3 - 2.5 - 3.5 - 4.5</td>
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<tr>
<td>40. How many hours per week did you usually do this activity?</td>
<td>&lt;1 / ≥1 but &lt; 2 / ≥2 but &lt; 3 / ≥3 but &lt; 4 / ≥4</td>
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<td></td>
<td>Time: 0.04 - 0.17 - 0.42 - 0.67 - 0.92</td>
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<tr>
<td>41. What was your typical rating of perceived exertion while doing this activity?</td>
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