CHARACTERIZING MECHANICAL EFFICIENCY IN PREGNANT WOMEN

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To those who are suffering from mental illness, this is for you. The struggle is real, the battle is hard, and some days, there is just no will to fight it. I have been there. I encourage you to try again. Keep fighting. Round up an army. This is not a battle that needs to be fought alone. Eventually, bad days become good days, and then good days become great days, and one day you find yourself not having to fight to feel good… you just do.

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PREFACE TO THESIS

This thesis contains data that was collected as part of the larger PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA) study. The PLACENTA study was funded by CIHR (MOP-142298), awarded to Dr. Kristi Adamo. Ethical approval was obtained from several Research Ethics Boards, including: the Ottawa Health Science Network (Protocol# 20160178-01H), the Children's Hospital of Eastern Ontario (REB# 16/68X), Hôpital Montfort (#LG-01-06-16), University of Ottawa (#H11-15-29), Queensway Carleton Hospital (File# 17-03). A portion of the thesis data was obtained from a sub-study conducted on non-pregnant controls which received ethical approval from the University of Ottawa (#H-11-17-190). I was funded through the Ontario Graduate Scholarship and the Canadian Graduate Scholarship – Masters (CIHR) in the first and second year, respectively.

The work contained in this thesis is my own, and I take full responsibility for its content. The PLACENTA study was designed by my supervisor, Kristi Adamo. I was involved in the creation of the study documents and obtaining ethical approval across several Research Ethics Boards. I was responsible for the data collection of over 40 women throughout the time spent in my MSc, which included leading over 100 study visits and attending 13 placenta pick-up calls. For the purpose of this thesis, the data from the exercise component for 20 women is presented.

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LIST OF ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
AEE	Activity energy expenditure
BMI	Body mass index
BMR	Basal metabolic rate
COM	Centre of mass
CON	Non-pregnant control group
CSEP	Canadian Society of Exercise Physiology
E_k	Kinetic energy
E_p	Potential energy
E_{tot}	Total energy
F	Force
GWG	Gestational weight gain
IOM	Institute of Medicine
MSK	Musculoskeletal
PREG	Pregnant group
REE	Resting energy expenditure
ROM	Range of motion
SOGC	Society of Obstetricians and Gynecologists of Canada
T1	Trimester 1, between 12 – 16 week gestation
T2	Trimester 2, between 24 – 28 week gestation
T3	Trimester 3, between 34 – 38 week gestation
TEE	Total energy expenditure
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen consumption
W_{ext}	External work
W_{int}	Internal work
W_{tot}	Total work

ABSTRACT

Pregnancy is an unique period in a woman's life in which her body undergoes rapid and drastic changes. Historically, physical activity was thought to be dangerous during pregnancy and women were recommended to avoid engaging in most physical activities. Mechanical efficiency, the ratio of external work and energy required to perform a task, is an important consideration when addressing the safety of physical activity, but also when defining recommendations to this population. Currently, there is limited literature that characterizes the change in mechanical efficiency across pregnancy. Of the available literature, suboptimal methodologies were employed, resulting in conclusions that conflict with what would be expected. The purpose of this thesis was to characterize mechanical efficiency across gestation and to compare with non-pregnant women. Women performed a standardized treadmill task in early, mid, and late pregnancy, and energy dynamics were measured. Results showed that energy requirements and external work performed increased over time, and that these were in relation to gestational weight gain. Pregnant women did not exhibit a change in the efficiency of performing a walking task. Overall, these results add to the current literature that supports women's engagement in physical activity during pregnancy.

CHAPTER 1: INTRODUCTION

Pregnancy is a unique time in a woman's life in which her body undergoes a myriad of rapid physiological, biochemical, and biomechanical changes to support the growth of the fetus (Girling, 2004). Many early published physical activity recommendations for pregnant women were based on little to no-empirical evidence and reinforced the notion that females were weak and frail (Downs, Chasan-Taber, Evenson, Leiferman, & Yeo, 2012; Maeder, 1977). Popular medical opinion from the late nineteenth century and into the first decades of the twentieth century was that pregnant women should use extreme caution to avoid fatigue and over exertion (ACOG, 1985). In today's society, most women are expected to continue the same occupational tasks and activities of daily living as they carry their baby to term, despite the increased physiological demands placed on the body. In addition, there is overwhelming evidence that women should remain physically active during pregnancy to promote optimal health for both mother and baby (Ferraro, Gaudet, & Adamo, 2012b; Hinman, Smith, Quillen, & Smith, 2015; Nascimento, Surita, & Cecatti, 2012).

Mechanical efficiency is the relationship between energy cost and external work completed for a given movement or task (Cavagna & Kaneko, 1977). Presently, there is limited literature examining how pregnancy affects the relationship between energy cost and external work, and thus mechanical efficiency. Of the literature available, findings seem contrary to what would be expected. For example, there are multiple reports of pregnant women using less oxygen for a given task and thus becoming more efficient movers as they progress throughout the gestational period (Byrne, Groves, Mcintyre, & Callaway, 2011; Clapp, 1989; Lotgering, Van Doorn, Struijk, Pool, & Wallenburg, 1991). This is in contrast to other scenarios of load carriage, such as wearing a backpack or carrying military equipment, which show a linear relationship

between external load carriage and energy cost, indicating that mechanical efficiency is unchanged when an external load is imposed (Grenier et al., 2012; T. Huang & Kuo, 2014; Taylor, Peoples, & Petersen, 2016). While pregnancy is distinct in that the increased 'load' is, in part, metabolically active tissue (Moya et al., 2014), the reports of improved mechanical efficiency in pregnancy are nonetheless surprising. Moreover, the methodology employed in previous work was suboptimal; variables known to affect efficiency – treadmill grade and walking speed (Cavagna, Saibene, & Margaria, 1963; Margaria, 1968) – were not kept constant throughout the gestational period. Clearly, additional work in this field of exercise physiology is warranted.

The literature review that follows will provide an overview of the physiological changes that occur with pregnancy, a historical overview of the beliefs about physical activity and pregnancy in contrast to current recommendations, the definition of mechanical efficiency, and finally, the gaps in the literature related to these aspects. The upcoming section will conclude with the objectives of the research undertaken.

CHAPTER 2: LITERATURE REVIEW

2.0 PHYSIOLOGICAL CHANGES IN PREGNANCY

Over nine months, a woman's body undergoes drastic changes in all body systems in response to the heightened demands of pregnancy and to support the growth and development of the fetus. These changes will be discussed in the proceeding subsections and are summarized in Figure 1.

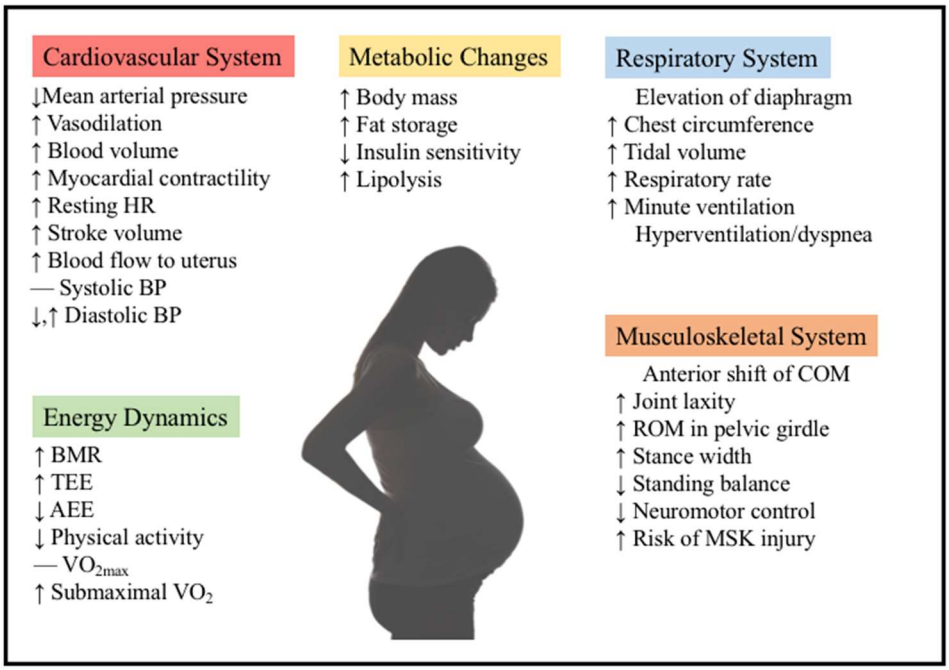

Figure 1. Overview of the physiological changes that occur in pregnancy. HR, heart rate; BP, blood pressure; BMR, basal metabolic rate; TEE, total energy expenditure; AEE, activity energy expenditure; VO_{2max}, maximal oxygen consumption; VO_2, oxygen consumption; COM, centre of mass; ROM, range of motion; MSK, musculoskeletal.

2.1 Cardiovascular System

Blood flow to the uterus and placenta is critical for the development and survival of the fetus. Insufficient blood flow, in response to poor placenta implantation, is one of the main etiologies of intra-uterine growth restriction (Huppertz & Peeters, 2005; Knöfler, 2010). Therefore, in order to ensure an ideal environment for fetal growth, the cardiovascular system undergoes several alterations; including becoming a low-resistance system and increasing blood volume. Vasodilation of the arterials, driven by increases in circulating nitric oxide, reduce arterial pressure (Granger, 2002). At the same time, there is an increase in blood volume and myocardial contractility which increases stroke volume (Girling, 2004; Robson, Hunter, Boys, & Dunlop, 1988). Resting heart rate (HR) also rises by ~15-20 beats per minute (San-Frutos, Engels, Zapardiel, & et al., 2011). As a result of the higher stroke volume and HR, there is a responsive increase in cardiac output during pregnancy (Granger, 2002; Robson et al., 1988). Overall, a quarter of the blood flow during pregnancy is directed to the uterus and placenta, with blood flow also enhanced to areas such as the breast, kidneys, and skin (Tan & Tan, 2013). Systolic blood pressure is relatively unaltered across gestation due to the opposing changes in stroke volume and mean arterial pressure; in contrast, diastolic blood pressure decreases until about 28 weeks of gestation, whereby it increases until term (Robson et al., 1988).

2.2 Respiratory System

Fetal growth causes the uterus to expand, and this, coupled with augmented abdominal pressure, causes elevation of the diaphragm. Concomitantly, the ligaments joining the ribs become more relaxed, driving an increase in chest circumference. The lower pressure and enlarged chest cavity reduce total lung capacity by 5% (Tan & Tan, 2013). Moreover, breathing dynamics are

distorted from these anatomical alterations. Tidal volume, the amount of air displaced within a cycle of normal inhalation-exhalation, and respiratory rate are increased in pregnancy, ultimately leading to a rise in minute ventilation. In late pregnancy, many women experience hyperventilation and dyspnea because of these changes (McAuliffe et al., 2002).

2.3 Musculoskeletal System

Pregnancy impacts the musculoskeletal system in many ways. Gestational weight gain, although essential for non-complicated pregnancy, occurs rapidly and causes an anterior shift in a woman's centre of mass (Foti, Davids, & Bagley, 2000; McCrory, Chambers, Daftary, & Redfern, 2010). Also, hormonal increases that support joint laxity as the fetus develops leads to a higher range of motion in the pelvic girdle and peripheral joints (Wolfe & Mottola, 1993). In response, women are required to assume different postures and gait parameters. Pregnant women have been shown to adopt wider stances, potentially to reduce postural sway, as a result of declined standing balance (J. Davies, Fernando, McLeod, Verma, & Found, 2002; Jang, Hsiao, & Hsiao-Wecksler, 2008). Other changes include a reduction in neuromotor control and decreased abdominal wall strength (McCrory et al., 2010) which, altogether, present challenges to pregnant women, such as a greater risk of falling (Dunning et al., 2003). Biomechanical analysis of gait in pregnancy highlight the adoption of reduced stride length (Branco, Santos-Rocha, Aguiar, Vieira, & Veloso, 2013; J. Davies et al., 2002; Forczek & Staszkiewicz, 2012), a slower walking speed (Byrne et al., 2011; J. Davies et al., 2002), and increased contact time with the ground (Branco et al., 2013; Forczek & Staszkiewicz, 2012). Others have shown greater activation and use of hip and ankle flexors and extensors in gait patterns, which could lead to overuse injuries or musculoskeletal dysfunction (Foti et al., 2000; Hagan & Wong, 2010).

2.4 Resting and Total Energy Expenditure

There is a wealth of literature indicating the increase in basal metabolic rate (BMR) from early to late pregnancy and between pregnant and non-pregnant populations. Lof *et al.* (2005) reported BMR values at 8, 14, and 20-week gestation to be similar to their pre-pregnant values, but higher in week 23 and 25 of gestation. Many others have shown a higher a BMR in mid or late-pregnancy compared to the non-pregnant state (Butte, Hopkinson, Mehta, Moon, & O'Brian Smith, 1999; Byrne et al., 2011; Gilmore et al., 2016; Melzer et al., 2010). Changes in BMR are highly correlated with body mass and gestational weight gain (Butte et al., 1999; Byrne et al., 2011; Gilmore et al., 2016; Lof et al., 2005; Melzer et al., 2010), whereby 60-70% of increase BMR are attributed to GWG. In a well-conducted study, where women were monitored for 24-hours in an indirect room calorimeter, Gilmore *et al.* (2016) measured BMR in women with low, adequate, and high gestational weight gain. Their results indicated that regardless of weight gained, BMR increased in proportion to GWG.

In the same study, Gilmore and colleagues assessed total energy expenditure (TEE) using double-labeled water and found that TEE in the third trimester was greater in women who were "high gainers" in comparison to women who were "low or ideal gainers". Not surprisingly, activity energy expenditure (AEE) and total physical activity decreased in both groups from early to late pregnancy. When comparing pregnant to post-partum, Butte *et al.* (1999) reported a higher TEE in pregnancy than 3-months and 6-months postpartum; maternal weight contributed to one-third of this difference. Of the three drivers of TEE – BMR, AEE and diet-induced thermogenesis – BMR contributes to ~75% of energy expenditure in pregnancy in both women who are normal weight (Butte et al., 1999) and in women with obesity (Most et al., 2018).

2.5 Impact on Aerobic Capacity

Safety concerns regarding maximal exercise testing in pregnant women have prevented many studies from exploring the topic of maximal aerobic capacity. However, the literature that is published suggests that pregnancy does not impact maximal aerobic capacity. Lotgering *et al.* (1991) conducted maximal aerobic testing on both the cycle ergometer and treadmill in pregnant women at 16, 25, and 35 weeks' pregnancy and 7 weeks' postpartum. In this case, maximal aerobic capacity was considered to be reached when the two of three of the following criteria were met: increase in O_2 consumption <5% in response to increased intensity; 2) increase in HR <5% in response to increased intensity; 3) a respiratory exchange ratio above 1.0. On the bike, exercise time and maximal power output were the same at all time points, except for at 35 weeks' gestation, where a 4% reduction in maximal power was present compared to postpartum values. However, $\dot{V}O_{2max}$ was not significantly different at any time point. Important to note is that an increase in resting $\dot{V}O_2$ values reduces the amount of O_2 available for exercise in pregnancy. Heenan *et al.* (2001) replicated these findings, showing that $\dot{V}O_{2max}$ (defined only by the criteria of volitional fatigue) was similar on the cycle ergometer between pregnant (35 weeks' gestation) and non-pregnant controls matched for age and height. Both these conclusions were derived from weight-supported assessments, which may undermine the impact of pregnancy on maximal capacity as they do not consider the impact of GWG. Lotgering *et al.* (1991) also assessed $\dot{V}O_{2max}$ on the treadmill and found similar results to those seen on the cycle ergometer. At 35 weeks' gestation, pregnant women had a 5% reduction in $\dot{V}O_{2max}$ compared to their postpartum values. Unfortunately, this study is the only one to date that has assessed maximal aerobic capacity.

Compared to maximal aerobic capacity, literature that has explored how submaximal intensities stress the pregnant body pregnant body's response to submaximal exercise is more

abundant. Changes in submaximal $\dot{V}O_2$ are dependent on the modality of exercise performed. For example, studies that have used weight bearing exercise have shown an increase in $\dot{V}O_2$ in pregnancy compared to nonpregnancy (Carpenter et al., 1990; Knuttgen & Emerson, 1974). Studies that employed a cycle ergometer modality have found less conclusive results, with some studies reporting an increase in $\dot{V}O_2$ (Carpenter et al., 1990; Pernoll, Metcalfe, Schlenker, Welch, & Matsumoto, 1975) but others indicating no change (Heenan, Wolfe, & Davies, 2001; Knuttgen & Emerson, 1974; O'Toole, 2003). As mentioned above, since the cycle ergometer is a weight-supported exercise, and therefore maternal weight would not be considered in the relationship, the latter findings better align with what is expected.

2.1 Physical Activity in Pregnancy: Then and Now

Historically, physical activity during pregnancy was considered unsafe and reinforced the idea that women were weak and frail (Downs et al., 2012). For example, the 1949 prenatal physical activity recommendations suggested activities such as housework, gardening, and walking up to 1-mile were safe, but sports participation was not; thus, women were told to abstain from such activities (Federal Security Agency and Social Security Administration, 1949). The initial physical activity guidelines set out by the American College of Obstetricians and Gynecologists (ACOG) in 1985 had recommendations which included: limiting vigorous exercise to 15 minutes or less and keeping a HR below 140 bpm (ACOG, 1985). Shortly after, a review in 1990 reported little evidence being available to support the benefits of physical activity (Snyder, 1990), suggesting that most benefits at the time were based on anecdotal reports or popular medical opinion. However, Synder discussed the available literature at the time, which mostly brought attention to potential risks of being active during pregnancy: how activity could lead to the restriction of uterine

blood flow, raise the risk of hyperthermia, and elicit fetal bradycardia. Ultimately, Synder concluded that given the scarce available literature at the time, more evidence was needed to conclusively accept or refute if physical activity was safe to perform during pregnancy.

Today, the guidelines for physical activity are more liberal and are becoming increasingly evidence-based. The joint recommendations from the Society of Obstetricians and Gynecologists Canada (SOGC) and the Canadian Society for Exercise Physiology (CSEP) for the Canadian physical activity guidelines during pregnancy have recently been updated, promoting greater physical activity than what was previously published in 2003 (G. Davies, Wolfe, Mottola, & MacKinnon, 2003). Now, the 2018 guidelines advise that all women with uncomplicated pregnancies achieve a minimum of 150 minutes of moderate-vigorous physical activity per week, and strongly encourage women to be active every day of the week (Joint SOGC/CSEP Clinical Practice Guideline, *in press to be released November 2018).* Moreover, there is now a wealth of literature supporting physical activity for the many benefits it provides to both mom and baby (see *2018 Physical Activity Guidelines for Americans*, 2018; Ferraro, Gaudet, & Adamo, 2012a; Szymanski & Satin, 2017). Unfortunately, like most Canadians, pregnant women fail to achieve these modest guidelines, with only 23% of women meeting the minimum amount (Gaston & Cramp, 2011). Paralleling this is the high rates of obstetric obesity and excessive GWG. Approximately 42% of Ontario women who gave birth to a baby between 2014-2016 were overweight or obese (BORN, 2016), and our recent systematic review indicates that nearly half of women exceed the recommended weight gain for their pre-pregnancy BMI class (Denize et al., 2018)

Longstanding concerns about the potential danger of activity in pregnancy still remain today (Guelfi et al., 2015), most likely are related to cultural beliefs or familial pressures (Symons

Downs & Hausenblas, 2004). Although there is increasing awareness among women, and greater promotion of physical activity as a healthful habit in pregnancy from healthcare providers, more evidence in support of this notion is warranted. In today's society, where women are achieving weight gain well above what is recommended, it is increasingly important to consider how this increased body mass may impact the efficiency of movement.

2.2 MECHANICAL EFFICIENCY

Efficiency is the ratio of the chemical and mechanical work performed and the metabolic energy used to complete a movement (Cavagna & Kaneko, 1977). Work can further be broken down into two major components: internal work (W_{int}) and external work (W_{ext}). Internal work pertains to the forces acting within a person such that the centre of gravity is not displaced in the external system. Examples of internal work are: *a)* maintaining an isometric contraction or *b)* equal and opposite movements of the body (Willems, Cavagna, Heglund, Umana, & Chelmsford, 1995). In contrast, external work is the sum of forces being exerted to move a person's centre of gravity relative to the external system (*i.e*. the ground). External work can be expressed as:

$$W_{ext} = Fs \cos\Phi$$

Where F is the sum of forces applied to the system, s is the displacement of the body, and Φ is the angle between F and s (Cavagna et al., 1963).

While it is advantageous to assess efficiency by looking at total work ($W_{tot} = W_{int} + W_{ext}$), measurement of internal work is complex, often captured through cinematography and biomechanical analysis (Fenn, 1930) and involves the consideration of how energy is transferred throughout the body. Previous reports suggest that energy transfer between limb segments can reduce W_{tot} by approximately 10% (Willems et al., 1995), and thus, excluding a measurement of

W_{int} will markedly reduce the overall result. Given the complexities of measuring W_{int}, research often includes only the measurement of W_{ext}. Measurement of W_{ext} alone can easily be done by looking at the sum of work that is performed after introducing a load to an otherwise unloaded movement (Cavagna & Kaneko, 1977). An example of this is walking on an incline on a treadmill. Since W_{ext} lacks the assumptions of within-body energy transfer that are involved when measuring W_{int}, one must consider how this limits the overall conclusions made from utilizing this methodology. Nonetheless, the study of W_{ext} has allowed scientists to identify fundamental concepts regarding human locomotion, such as the pendulum pattern humans exhibit in bipedal walking (Cavagna & Kaneko, 1977), and provides insight into how the efficiency of locomotion may differ across the lifespan, body masses, or in situations of external load carriage.

The Efficiency of Human Locomotion

The efficiency of human locomotion is different depending on the type of locomotion, for instance walking compared to running. This difference in efficiency is related to the different mechanics of movement during the walking and running patterns exhibited in human locomotion. Throughout step cycles, there are exchanges of potential (E_p) and kinetic (E_k) energy; in walking, this exchange happens in opposition (E_p increases at E_k decreases), whereas in running the changes in E_p and E_k are simultaneous (Cavagna et al., 1963). During walking, efficiency is between 0.20 and 0.40. The relationship between speed and efficiency in walking is an inverted U, such that the greatest efficiency is observed at intermediate walking speeds (~5 km/h). In contrast, the efficiency of running is ~ 0.40 and increases in a linear fashion as speed increases (Cavagna & Kaneko, 1977). Accordingly, the faster one runs, the more efficient one becomes. The stretch-shortening

cycle of the tendons allows the simultaneous exchange between E_p and E_k; this way the energy can be stored and released quickly, creating a more efficient movement pattern.

Mechanical efficiency, which pertains only to the W_{ext} executed, requires that there is net W_{ext} completed for accurate assessment. For example, examining walking or running on a level ground is not possible and requires the W_{ext} to be measured against an applied load. Throughout a step cycle, both positive and negative work are performed; on level ground, these forces cancel each other amount resulting in no positive work being completed (Margaria, 1968). This relationship between efficiency and external work has led to modern literature utilizing an incline treadmill to apply an external load to assess the mechanical efficiency of locomotive patterns.

Scenarios of external load carriage have found minimal influence of an external load on mechanical efficiency. For example, the use of military protective equipment is one area that has explored this topic in detail (Taylor et al., 2016). Huang & Kuo (2014) reported a linear increase in net metabolic rate when carrying a heavier backpack, which was driven by greater vertical and horizontal ground reaction forces. A linear relationship was also shown between backpack weight and W_{ext}. The same linear relationships between energy cost, mechanical work and efficiency have been exhibited in situations of load carriage in soldiers. Comparing between an unloaded condition and two loaded conditions, there was a higher energy cost, and greater W_{ext} as the load increased from body mass to an additional 24 kg and 38 kg (Grenier et al., 2012). Overall, these changes in energetics did not influence mechanical efficiency in walking.

Findings similar to those previously mentioned have been found when comparing people who are overweight and obese to those of normal weight. In children, Huang *et al.* (2013) found that children who were overweight had a similar mechanical efficiency of walking, despite having a higher metabolic cost, to children who were of normal weight (Huang, Chen, Zhuang, Zhang, &

Walt, 2013). In contrast to these findings, adult women with obesity displayed less efficient cycling patterns than their normal weight equivalents (Lafortuna et al., 2008). The same women had a greater cost of transport than women of normal weight when walking on a treadmill at a speed of 1.3 mph with an incline of 4%. Presumably, the increased cost can be related to their body mass increasing the amount of W_{ext} required to complete the task. Another potential explanation for this could be associated to the location of body mass, whereby persons with obesity have extra mass on their limbs leading to a disproportionate increase in work for walking (Saibene & Minetti, 2003)

The Efficiency of Walking in Pregnancy

To date, few studies have addressed the change in mechanical efficiency throughout pregnancy. The augmented energy demands of pregnancy mean that women are working at higher relative percentages of their maximal capacity, therefore, it is important to consider if and how changes in efficiency may impact this relationship. Two early studies concluded that pregnancy led to an improved movement efficiency in comparison to that seen before conception and early pregnancy (Clapp, 1989; Lotgering et al., 1991). More recently, this relationship was considered in a cohort of pregnant women with obesity. Consistent with past findings, Byrne and colleagues (2011) also concluded that pregnant women became more efficient movers in late pregnancy. In all scenarios, methodological flaws exist that raise concerns about the validity of their findings. For example, Clapp (1989) and Byrne *et al.* (2011) both adjusted for body mass to reach their final conclusions. Since GWG is comprised of metabolically active tissue, adjusting for body weight may lead to an inaccurate estimation of oxygen consumption. Lotgering *et al.* (1991) and Byrne *et al.* (2011) concluded that efficiency was improved due to a marked decrease in oxygen consumption for the walking task; however, in both cases, the speed of the walking task was not

constant throughout pregnancy. Additionally, one study utilized a treadmill protocol which was kept at a 0% grade, further questioning the validity of conclusions (Byrne et al., 2011). The findings from these studies are also in conflict with the other literature examined thus far, which conclude that excess weight – due to either body mass or external load – does not impact overall mechanical efficiency. Given the ongoing consideration of the safety of exercise in pregnancy, further studies in this area are warranted.

2.3 Objective of Thesis

The objective of this thesis was to explore mechanical efficiency across the entire gestational period in healthy women with uncomplicated pregnancies. This objective had two specific aims:

1. To characterize mechanical efficiency in pregnant women during light-moderate physical activity.
    a. To characterize mechanical efficiency in early, mid, and late pregnancy.
    b. To compare mechanical efficiency in pregnant women and non-pregnant women.
2. To explore the relationship between mechanical efficiency and health-related parameters, including habitual physical activity and GWG.

CHAPTER 3: METHODS

3.1 ETHICAL APPROVAL

This thesis data capitalized on the infrastructure and participant pool provided by the CIHR-funded trial, the PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA) study (MOP-142298). The protocol was reviewed and approved by several institutional research ethics boards across Ottawa (Appendix A**)** including: the Ottawa Health Sciences Network (REB# 20160178-01H), the Children's Hospital of Eastern Ontario (REB# 16/68X), Hôpital Montfort (#LG-01-06-16), University of Ottawa (#H11-15-29), and Queensway Carleton Hospital (File# 17-03). The sub-study of non-pregnant controls was approved by the University of Ottawa (REB# 11-17-190). Participants provided written, informed consent before participation.

3.2 STUDY PARTICIPANTS

Pregnant women (PREG) and non-pregnant women (CON) were recruited in the Ottawa region (Ontario, Canada). Pregnant women were required to meet the following inclusion criteria: 18-40 years old; pre-pregnancy BMI between 18.5 – 29.9kg/m^2; equal or less than 28 weeks of gestation at time of recruitment; weight stable ± 5kg for at least 6 months prior to pregnancy; carrying a singleton fetus; and cleared for participation by their health care provider, thus not present with contraindications to exercise. Women who had pregnancy complications such as pre-pregnancy insulin-treated diabetes, untreated thyroid disease, or hypertension requiring medication at the time of recruitment, planned to have their child adopted, or were unable to communicate in English or French were excluded. Recruiting women prior to conception was not feasible; in light of this, healthy women matched for age, body mass and BMI were sought to provide a non-pregnant comparator.

3.3 DATA COLLECTION

3.3.1 Pregnant Women

Pregnant women visited the lab on three separate occasions between week 12-16, week 24-28, and week 34-38 of gestation. All laboratory visits occurred in the morning between 6h00 and 10h00, and after an 8-hour fast. At all visits, maternal height was measured, and maternal weight was recorded. Participants were then given a standardized snack (370 kcal, 7g fat, 74g carbohydrate, 5g protein. Prior to the exercise test, resting energy expenditure was measured for 20 consecutive minutes. A modified HALO submaximal exercise test (Appendix B) was performed on a calibrated treadmill (see Appendix C). This protocol has previously been shown to be safe in the pregnant population (K. E. Brett, Ferraro, Holcik, & Adamo, 2015). In brief, all women began with a 4 minute warm-up at a speed of 3.2 km/h and a 0% grade. Then, keeping the speed constant at 3.2 km/h, the grade increased by 2% every 3 minutes (hereinafter referred to as a Stage), for a total of 21-minutes (7 stages total). A 2 minute cool down, performed at a speed of 1.6 km/h and 0% grade, was completed at the end of the test. Participants were instructed to keep conversation to a minimum during the test and to avoid using the hand rails on the treadmill for support. Heart rate was recorded in 30-second intervals during rest and exercise (Polar V800, Polar Electro Canada). Adverse events such as musculoskeletal injury, cardiac events, falls and signs of physiological stress (such as dizziness, leg pain, chest pain, nausea, shortness of breath) were recorded on the data collection sheet. .

3.3.2 Control Group

Women in the control group followed a similar protocol as those in the pregnant group. A small subset of non-pregnant women (n=3) completed the exercise trial at two-time points, three months apart, to assess if any change in responses were subject to natural variations over time.

3.3.3 Anthropometric Measures

Maternal height and body mass were measured by trained research personnel, following the validated CSEP-PATH methodology (CSEP, 2013) at all study visits. In brief, height (cm) was measured using a stadiometer (HR-200 Wall-Mounted Height Rod, Tanita Corporation of America Inc., Illinois, USA) in duplicate, and reported to the nearest 0.1cm. The participant removed footwear and was instructed to stand with their feet together, the heels, buttocks and upper back touching the wall. With their arms hanging at their sides and head looking straight ahead, the participant was asked to exhale, which after the headboard of the stadiometer was lowered until it touched the top of the head. The measurement was recorded after the participant stepped away. Body mass (kg) was measured using an electronic scale (BWB-800 Doctors Scale, Tania Corporation of America Inc., Illinois, USA**)**, while participants were barefoot and wearing light clothing. After zeroing the scale, the participant stood in the centre of the scale and remained still. When a stable reading was obtained, body mass was recorded to the nearest 0.1kg. GWG was calculated by measured body mass at T3 – self-reported pre-pregnancy body mass, and then categorized as inadequate, adequate, and excessive as defined by the BMI-specific IOM recommendations shown in Table 1.

Table 1: Institute of Medicine 2009 Guidelines for Gestational Weight Gain

Pre-Pregnancy Weight Class	Pre-Pregnancy BMI (kg/m2)	Total Weight Gain (kg)
Underweight	<18.5	12.7-18.1
Normal weight	18.5-24.9	11.3-15.9
Overweight	25.0-29.9	6.8-11.3
Obese	>30.0	5.0-9.1

3.3.4 Metabolic Measurements

Participants were fitted with a face mask connected to a pull-through open circuit field metabolic system (FMS Field Metabolic System, Sable Systems International, Las Vegas, USA) to measure oxygen consumption and carbon dioxide production. The flow rate was set at 100L/min during rest and 200L/min during exercise. The flow rate was validated using the Life Wind flow meter (Polycontrols Inc, QC, Canada**)**. Barometric pressure (P_B), and the composition of inspired (f_i) and expired (f_e) air were measured from a 250mL/min subsample using a field metabolic system (Sable Systems International Inc., Las Vegas, USA). Water vapour pressure (WVP) of f_i and f_e air was measured using a flow-through water vapour analyzer (RH-300 Water Vapour Analyzer, Sable Systems International Inc., Las Vegas, USA). Temperature (T) and relative humidity were controlled at 23°C and 20%**,** respectively. The system was calibrated with a standard mixture of gas (Mpri Oyxgen 8% N2 Bal 200Sz, Linde Canada, ON, Canada) prior to each trial (for an in-depth calibration, see Appendix C).

Flow rate obtained from the Life Wind was converted to Standard Temperature, Pressure, Dry (STPD) using the combined gas laws as follows:

$$FR = \frac{T_{Standard} \times P_B}{T_{Ambient} \times P_{Standard}}$$

$$FR = \frac{273K \times (P_B\ kpa - WVP\ kpa)}{296K \times 101.3kpa}$$

3.3.5 Measure of Habitual Physical Activity

Habitual physical activity was measured using an Actical® accelerometer over seven days after each visit in the PREG group. The Actical® is a small omni-directional accelerometer that

measures and records time-stamped acceleration. Data was collected in 60-second epochs, providing raw data in counts per minute (cpm). Minutes of MVPA were counted when performed in bouts of at least 10-minutes. Participants wore the accelerometer on their right hip, and were instructed to only remove the device for water-based activities (ex. swimming and bathing) and sleeping. Since accelerometers are limited in capturing some activities such as swimming and cycling, participants completed a physical activity journal to capture these data. A composite score was used in the final analysis to assess physical activity levels in the cohort. Activity levels were compared to the Canadian Physical Activity Guidelines, which currently recommend a minimum of 150 minutes of moderate-vigorous physical activity (MVPA) per week, accumulated in at least 10-minute bouts. Currently, there is no consensus on Actical processing criteria (i.e., cut points, number required for valid days, epoch lengths) leading to large heterogeneity in study design and analysis (Sattler et al., 2018). Adding to this is the different perceptional level of physiological strain that a pregnant woman experiences during exercise. We chose to analyze our PA data consistent with the Canadian Health Measures Survey methodology (Colley, 2012). Despite the lack of validity of using an accelerometer in pregnant women, the Actical provides a superior and objective approach to physical activity measurement than self-reported questionnaires (Brett, Wilson, Ferraro, & Adamo, 2015).

3.4 DATA ANALYSIS

3.4.1 Energy Expenditure

Oxygen consumption ($\dot{V}O_2$) was calculated with the following equation:

$$\dot{V}O_2(\frac{L}{\text{min}}) = Flow\ Rate, STPD\ \times\ (fiO_2 - feO_2)$$

where flow rate is in L/min, fiO_2 and $fiCO_2$ are the fractions of the measured gas species in the ambient air; and feO_2 and $feCO_2$ are the fractions of the measured gas species leaving the mouthpiece.

Energy expenditure (EE) was calculated using the thermal equivalents of $\dot{V}O_2$ for the non-protein respiratory quotient at each stage of the exercise protocol with the following equation:

$$EE\ (kcal) = \dot{V}O_2\ \times\ RQ\ Caloric\ Equivalent \times\ Time$$

where $\dot{V}O_2$ is oxygen consumed in L/min and time is the length of the stage in seconds.

The area under the curve (AUC) was calculated for 20 minute at rest and 21 minutes during the exercise protocol using the trapezoid Riemann sum. Resting energy expenditure (REE) is considered here as the energy expended over the course of the 20 minute resting period. Activity energy expenditure (AEE) is the energy expended over the 21-minute exercise test.

3.4.2 Work Calculations

Total external work (W_{ext}) completed on a calibrated treadmill was calculated from participant body mass, and vertical distance travelled using the following equation:

$$W_{ext}\ (kcal) = [Body\ Mass \times\ Speed\ \times\ Time\ \times \sin(\theta)]\ \times\ 0.000234$$

where body mass is participants mass in kg, speed is treadmill speed in in $m*min^{-1}$, time is in minutes, θ is the measured angle of the treadmill at each stage, and .000234 is the conversion factor from kgm to kcal.

3.4.3 Efficiency Calculation

Mechanical efficiency (ME) was calculated using W_{ext} and AEE, as follows:

$$ME\ (\%)\ = \frac{W_{\ Ext}}{AEE}$$

where W_{Ext} is the total work completed over the exercise test in kcal, and AEE is the energy expended over the exercise test in kcal.

3.5 STATISTICAL ANALYSIS

Statistical analysis was performed on SPSS, version 24 (SPSS Inc., Armonk, NY, USA). Statistical significance was set at $P < 0.05$ (two-tailed). The assumption of normality was assessed using the Shapiro-Wilk test. When data assumed normality, repeated measures analysis of variance (ANOVAs) were conducted, and if the assumption was violated, Friedman's test was used for when comparing variables across pregnancy (T1, T2, and T3). To compare the control group and the pregnant group, Student's independent *t*-tests (normally distributed) and Mann Whitney U tests (non-normal distribution) were used between control and each of the trimester time points. Pearson's correlation coefficients were run to determine relationships among variables.

3.5.1 Sample Size Calculation

A sample size calculation was conducted on preliminary $\dot{V}O_2$ data ($N = 5$) to determine adequate statistical power ($\beta = .80$). The minimum sample size to yield a statistical significant result ($p < .05$) and a minimum difference in VO_2 across time points of 300 mL/min was indicated at 5 subjects for within-subject comparison (ANOVAs) across the trimesters, and 14 subjects, per group, for between-subject comparison between pregnant- and non-pregnant women (student *t*-tests).

CHAPTER 4: RESULTS

Participant characteristics for both PREG and CON are described in Table 2. As designed, women in CON were similar in age, body mass, and BMI to the PREG group ($P > 0.05$). Eleven pregnant women and ten non-pregnant women participated in the study. One pregnant woman requested to terminate the exercise test early at the T3 time point due to hip pain, and therefore was excluded from the analysis. Other than the one participant to complain of musculoskeletal pain, the other ten women completed the exercise test with no adverse events. Moreover, no adverse events were reported in their physical activity journals on the days the participants wore the Acticals. A total of twenty participants are included in the analysis, with ten women in each cohort.

Table 2. Participant characteristics for CON and PREG groups

	CON	PREG		
		T1	T2	T3
Age, years	30.4 ± 3.7	31.9 ± 3.7	–	–
Gestational age, weeks	–	15.4 ± 0.9	26.4 ± 1.1	35.3 ± 1.1
Body mass, kg	63.6 ± 7.2	65.7 ± 9.8	71.6 ± 10.2	77.3 ± 11.9
BMI, kg/m^2*	23.4 ± 1.87	22.1 ± 1.9	–	–
Resting HR, bpm	66.6 ± 14.7	74.1 ± 6.9	79.1 ± 7.5	80.5 ± 7.9
Resting RQ	0.88 ± 0.06	0.85 ± 0.08	0.83 ± 0.07	0.85 ± 0.07

Values are presented as mean ± SD. CON = non-pregnant women, N=10; PREG = pregnant women, N=10; BMI = body mass index; RQ = respiratory quotient; *Based off of self-reported body mass and measured height at initial study visit

4.1 OBJECTIVE 1

4.1.1 Resting Measurements

Figure 2 presents the REE in CON and PREG. Compared to the non-pregnant state, REE was higher in pregnancy at T2 ($P = 0.04$) and T3 ($P = 0.01$), but not T1, time points ($P = 0.45$). The change in REE across trimesters within PREG increased by 5.3 ± 1.13 kcal at T3, however, this increase was not significant ($P = 0.06$). As shown in Table 2, resting RQ was similar between

pregnant and non-pregnant groups, and across pregnancy (all $P > 0.05$). Resting HR values increased from the beginning to end of pregnancy ($P = 0.03$), and were higher than CON at T2 ($P = 0.03$) and T3 ($P = 0.02$) time points (see Table 2).

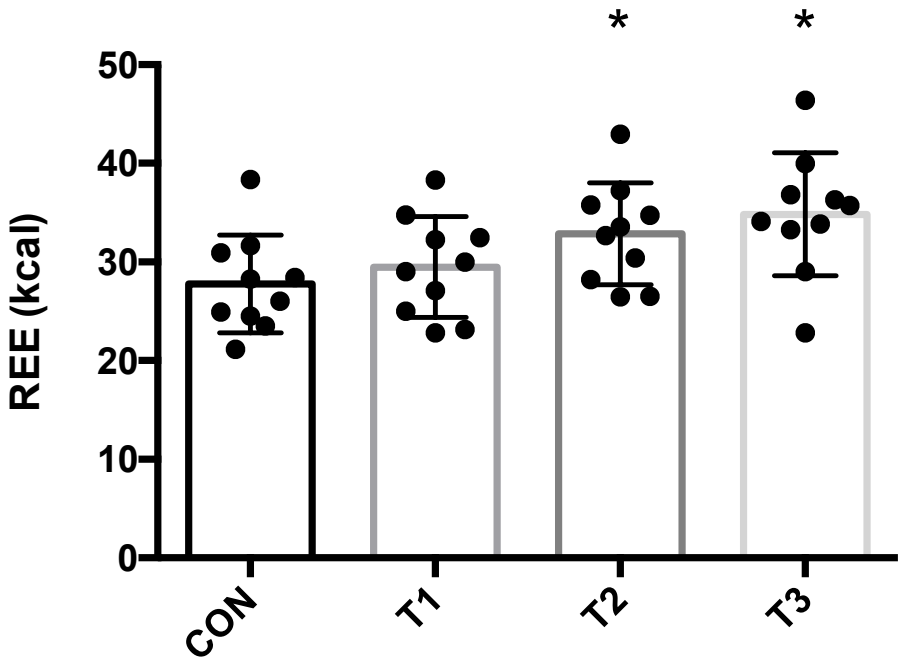

Figure 2. Energy expenditure (kcal) over the 20-minute resting period. CON, non-pregnant women, N=10; T1, early pregnancy, N=10; T2, mid-pregnancy, N=10; T3, late pregnancy, N=10. *p < 0.05, significantly different from CON; error bars represent SD.

4.1.2 Energy Expenditure and Work Completed

Figure 3 displays the difference in AEE across PREG and in comparison to CON. AEE increased across pregnancy ($P = 0.02$). Similarly, W_{ext} increased from early, mid, to late pregnancy (Figure 4). AEE was higher in PREG compared to CON at T2 ($P = 0.02$) and T3 ($P = 0.01$) time points. W_{ext} was similar between CON and women at T1 ($P = 0.65$) and T2 ($P = 0.07$), however, more W_{ext} was completed by PREG at T3 ($P = 0.01$). Both differences in AEE and W_{ext} were highly correlated with body mass (Figure 5 and Figure 6, respectively). Additional correlations between anthropometric, REE and AEE are presented in Table 3.

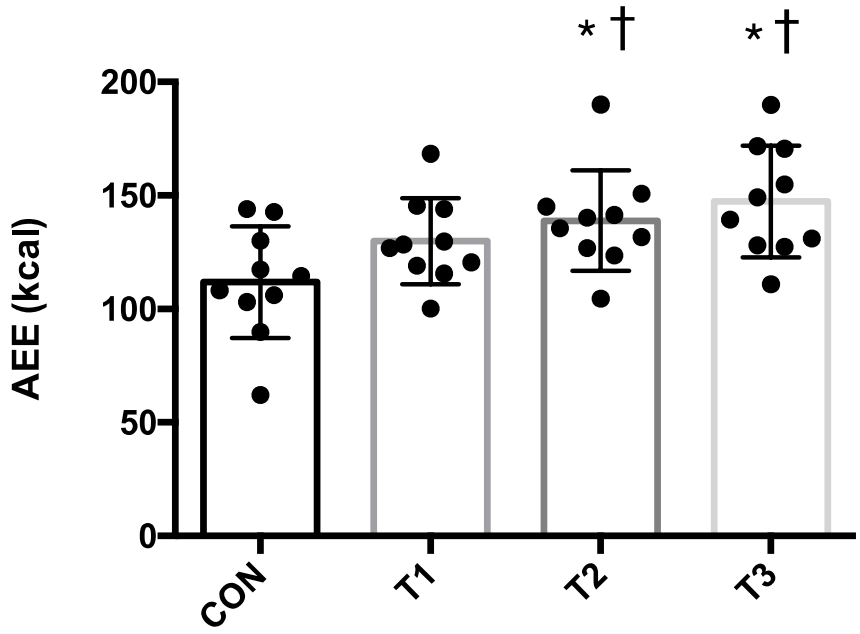

Figure 3. Energy expended (kcal) over the 21-minute standardized exercise task. CON, non-pregnant women, N=10; T1, early pregnancy, N=10; T2, mid-pregnancy, N=10; T3, late pregnancy, N=10. *p < 0.05, significantly different from CON; †p < 0.05, significantly different from T1; error bars represent SD.

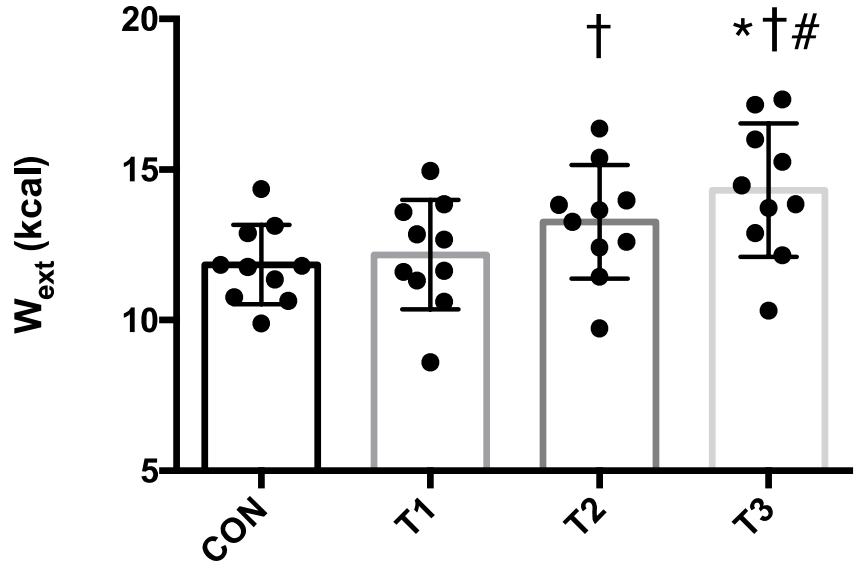

Figure 4. Total external work completed over the 21-minute standardized exercise task. CON, non-pregnant women, N=10; T1, early pregnancy, N=10; T2, mid pregnancy, N=10; T3,

late pregnancy, N=10. *p < 0.01, significantly different from CON; †p < 0.01, significantly different from T1; # p < 0.01, significantly different from T2; error bars represent SD.

Table 3. Pearson's correlation for anthropometric measurements, resting and activity energy measures

	pBMI	GWG	REE_{T1}	△REE	AEE_{T1}	△AEE
Age	0.259	**-0.671**	0.429	-0.397	0.320	-0.531
pBMI		0.299	0.339	0.218	0.339	0.167
GWG	0.299		-0.147	0.605	-0.147	**0.844**
REE_{T1}	0.339	-0.147		-0.603	**0.768**	-0.221
△REE	0.218	0.605	-0.603		-0.169	0.588
AEE_{T1}	**0.735**	0.136	**0.768**	-0.16		-0.071
△AEE	0.167	**0.844**	-0.221	0.588	-0.071	

Bolded font indicates $P < 0.05$. pBMI = pre-pregnancy BMI; GWG = gestational weight gain; △REE = change in resting energy expenditure from T1 to T3; REE_{T1} = resting energy expenditure at T1; AEE_{T1} = activity energy expenditure at T1; △AEE = change in activity energy expenditure from T3 – T1; ME_{T1} = mechanical efficiency at T1

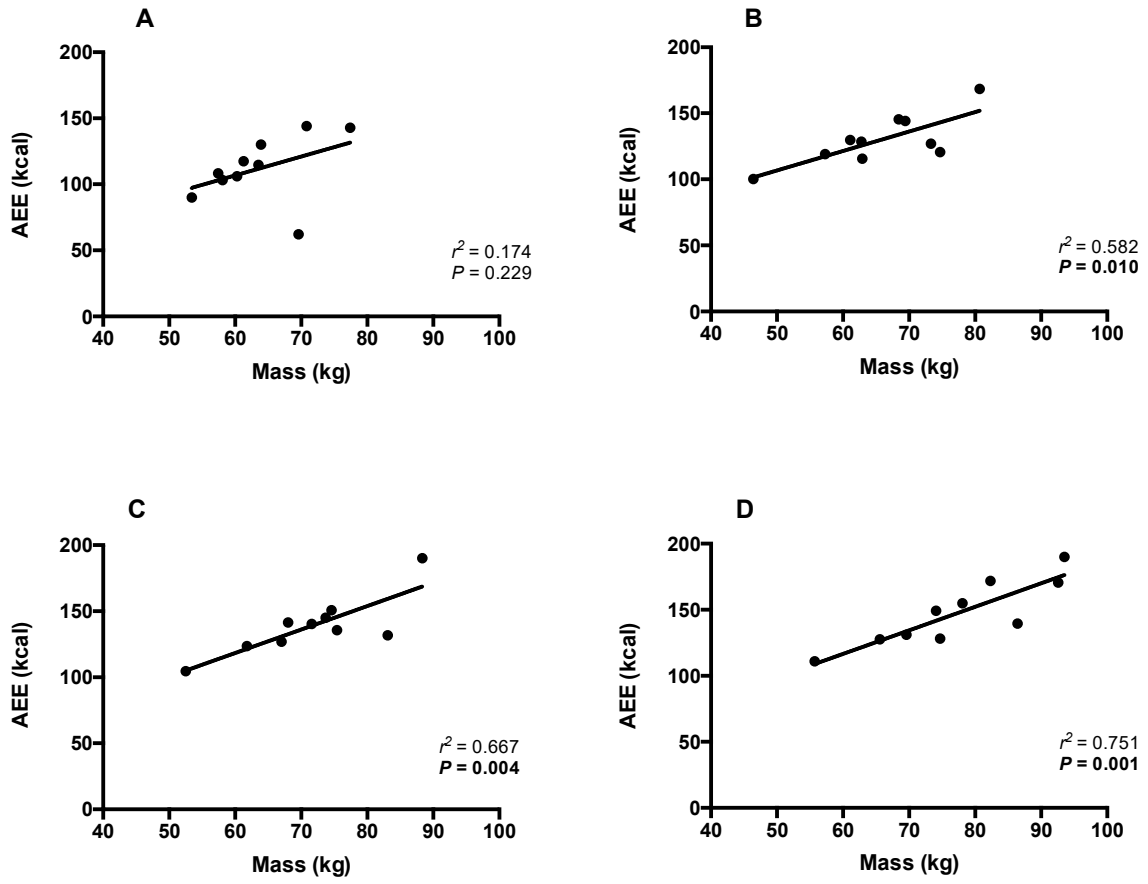

Figure 5. Relationship between activity energy expenditure and body mass. A: Control, N=10; B: Pregnant T1, N=10; C: Pregnant T2, N=10; D: Pregnant T3, N=10; AEE, activity energy expenditure; bold font indicates significant relationship between AEE and mass p <0.05

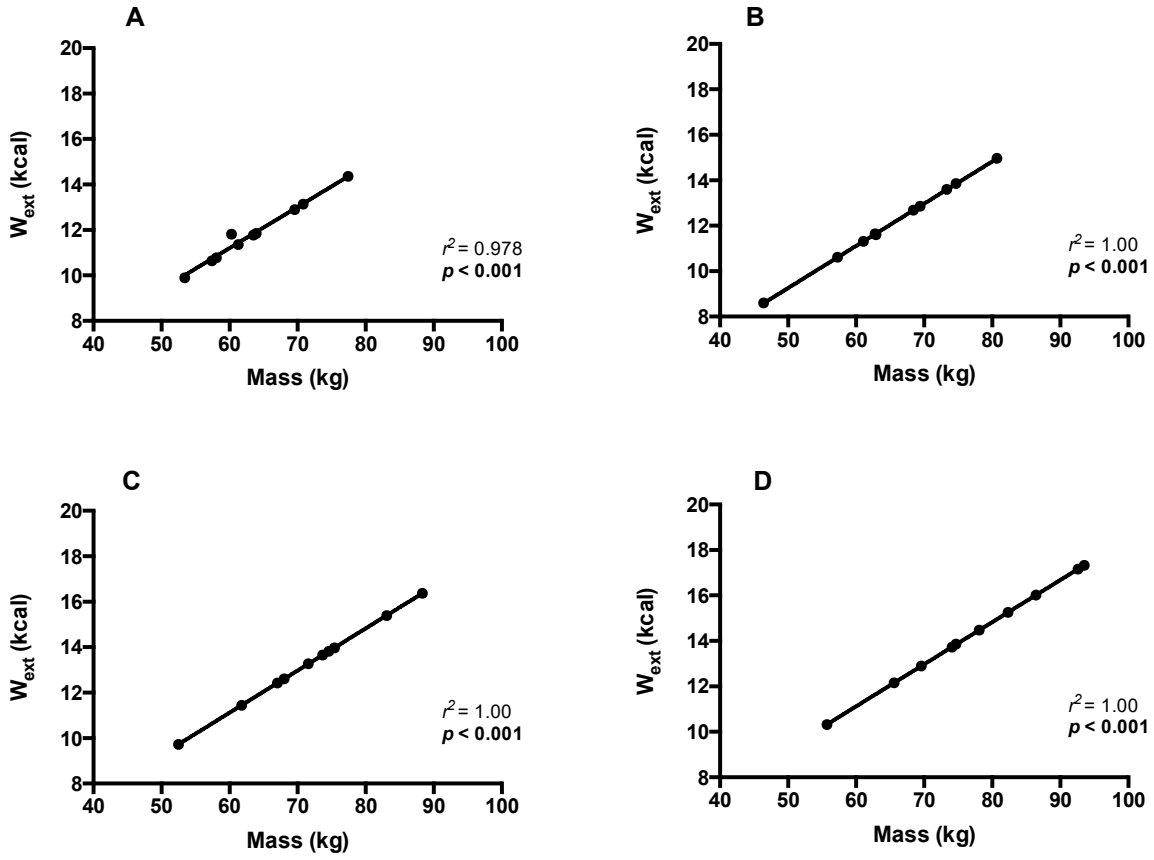

Figure 6. Relationship between external work and body mass. A: Control, N=10; B: Pregnant T1, N=10; C: Pregnant T2, N=10; D: Pregnant T3, N=10; AEE, activity energy expenditure; bold font indicates significant relationship between AEE and mass p <0.05

4.1.3 Increase in Heart Rate

Increase in HR, from rest to maximum HR achieved at the end of the 21-minute exercise test, was minimal in each participant (Figure 7) and the magnitude of the increase in HR was not significantly different across pregnancy trimesters nor between PREG and CON, at any time point (all $P > 0.05$). Maximum HR achieved during the test was higher in T3 (148 ± 11 bpm) compared to T2 (138 ± 11 bpm; $P < 0.001$) and T1 (136 ± 14 bpm; $P < 0.001$). Compared to CON (125 ± 10

bpm), PREG attained higher HR at Stage 7 in T2 ($P = 0.02$) and T3 ($P < 0.001$), but not T1 ($P = 0.07$).

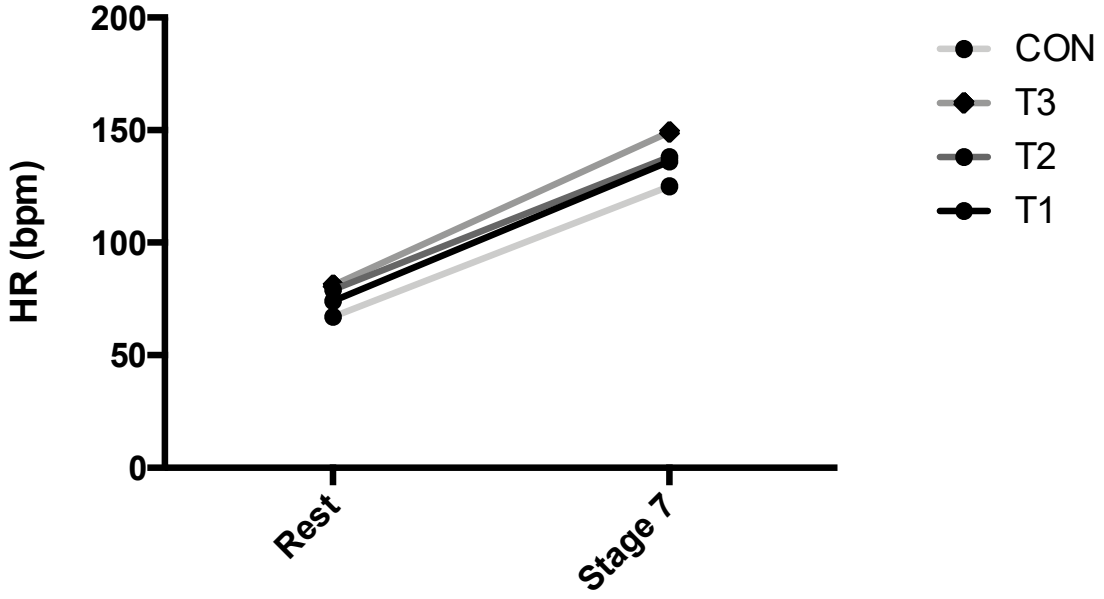

Figure 7. Change in HR from rest to stage 7 of the walking task. CON, non-pregnant women, N=10; T1, early pregnancy, N=10; T2, mid-pregnancy, N=10; T3, late pregnancy, N=10; HR, heart rate; Stage 7, the last stage of the 21-minute exercise test; no significant differences between groups indicated

4.1.4 Change in Mechanical Efficiency

On average, mechanical efficiency was unaffected by pregnancy (Figure 8), with efficiency to perform the walking task the same at all stages in pregnancy ($P = 0.74$). Of the ten women: six women had no change in the efficiency; one woman had a 1% increase in efficiency at T3; one woman had a 1% decrease in efficiency at T3; and, two women had a 1% decrease in efficiency in T2, but equal efficiencies at T1 and T3. Compared to CON, mechanical efficiency was lower in PREG at all three time points (T1: $P = 0.04$; T2: $P = 0.02$; T3: $P = 0.02$).

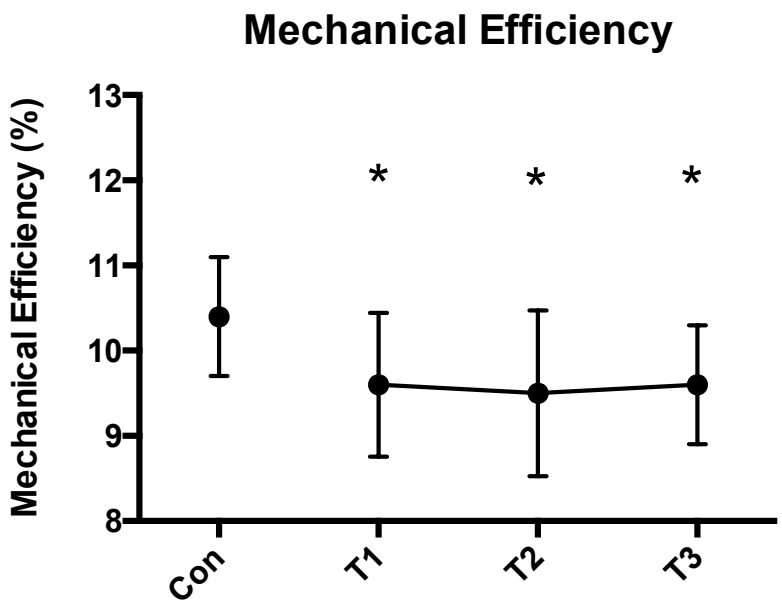

Figure 8. Mechanical efficiency of treadmill walking in pregnancy and in non-pregnant controls. CON, non-pregnant women, N=10; T1, early pregnancy, N=10; T2, mid-pregnancy, N=10; T3, late pregnancy, N=10. *$p < 0.05$, significantly different from CON; error bars represent SD.

4.2 OBJECTIVE 2

Our cohort of pregnant women was inactive when assessed according to the Canadian Physical Activity Guidelines. On average, women participated in 12.3 ± 10.4 min/day at T1, 7.6 ± 6.6 min/day at T2, and 7.2 ± 11.0 min/day at T3 of moderate physical activity. All ten women entered pregnancy with a BMI classified as normal, and therefore, the recommended weight gain was between 11.3 – 15.9 kg according to the IOM guidelines. Of the ten women, five gained within the GWG recommendations, one exceeded, and four did not achieve the recommended weight gain. Average GWG was 11.6 ± 3.8 kg, with a range between 6.3 – 17.9 kg. Figure 9 displays these data.

The two women who experienced a small decrease in efficiency in T2 did not have unique patterns of GWG in comparison to women who had no change in efficiency. Similar GWG patterns

were identified between the women who had a slight increase in efficiency at T3. However, the one woman who had a 1% reduction in efficiency was the only participant who experienced excessive GWG. The individual evaluation of physical activity does not suggest any significant relationships between physical activity and efficiency. The two women that were inactive in T1 remained inactive throughout their pregnancy. Except one, all other women reduced their activity levels over the course of pregnancy (range: 4.2 – 37.7 min/week reduction). The one woman who increased her activity levels from T1 to T3, was also the woman to experience the small increase in efficiency at T3.

Although the individual trends display a potential relationship, overall, the relationship between mechanical efficiency, physical activity, and GWG present no strong patterns in this cohort of women. Therefore, it can be inferred that neither physical activity level nor GWG impacts the efficiency of this walking task.

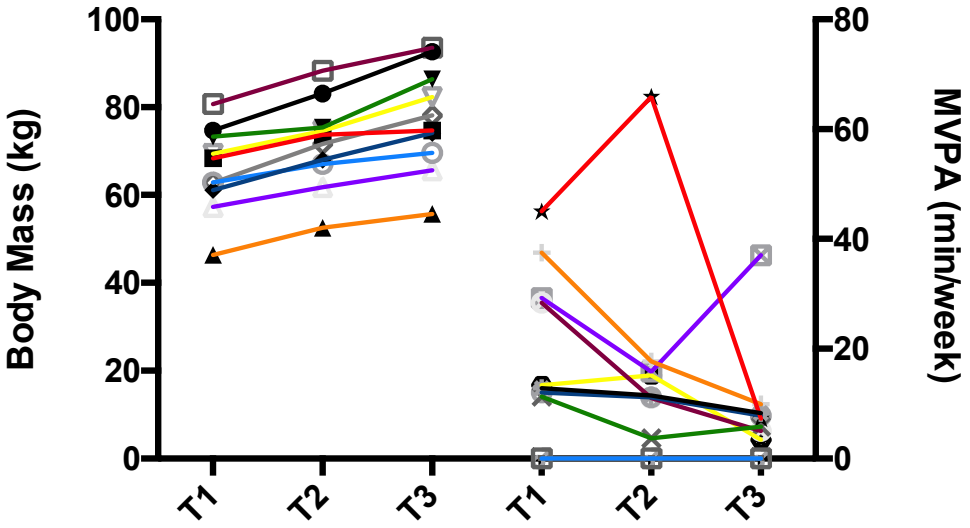

Figure 9. Physical activity patterns and GWG in pregnant women across the three trimesters of pregnancy. MVPA, moderate-vigorous physical activity measured over a 7-day period; T1, early pregnancy, N=10; T2, mid-pregnancy, N=10; T3, late pregnancy, N=10; *p < 0.001, significantly different from T1; each colour represents an individual participant

CHAPTER 5: DISCUSSION

The overall goal of this thesis was to explore the impact of pregnancy on mechanical efficiency, by specifically examining the relationship between energy demand and W_{ext} completed for a standardized walking task. The results indicate that women expend more energy to accomplish the same task and that more W_{ext} is completed for the task as they progress from early to late gestation. These changes increased in proportion to each other, resulting in no change in mechanical efficiency across pregnancy (Figure 8). Similarly, pregnant women had higher energy expenditure and W_{ext} rates than non-pregnant women in the second and third trimester. No difference in efficiency was found when comparing the first trimester data to the non-pregnant controls; this can most likely be attributed to the groups having similar body masses. Statistical analysis suggested a less efficient movement pattern in pregnant women compared to non-pregnant controls, although the overall change was minimal (~1%); this difference being equivalent to about a 15 kcal higher energy demand for pregnant women to complete the exercise task. Given that pregnant women expend approximately 400 kcal extra per day compared to non-pregnant women (Butte, Wong, Treuth, Ellis, & Smith, 2004; Durnin, 1991), this small increase in energy requirement likely has little impact on overall daily energy dynamics. It is more plausible that this small difference in efficiency is related to inter-individual variations in movement patterns (Cavanagh & Kram, 1985; Pedotti, 1977; Simonsen & Alkjaer, 2012), which are exploited in our study design. Recognizing the complexities and challenges associated with recruiting women prior to conception, but also the importance of having a non-pregnant physiological comparator, we opted to use an independent non-pregnant control group. The first visit during pregnancy was not until the 12 – 16th week of gestation, therefore, the addition of a non-pregnant comparator allows us to confirm that our observations seen in this study are truly related to pregnancy. Specifically,

The control group indicated that responses observed at the T1 visit are similar to the non-pregnant state. Overall, the linear increases in energy expenditure and W_{ext} exhibited in the pregnant group in mid- and late-pregnancy, in direct relation to their GWG (Figure 4 and Figure 5), and the ensuing unchanged mechanical efficiency, suggests that pregnancy does not have an impact on overall mechanical efficiency for a standardized walking task. Importantly, no adverse events occurred throughout the study period in the ten pregnant women, supporting the idea that moderate physical activity can be safely performed in pregnancy.

A secondary objective of this thesis proposal was to examine if GWG or physical activity might alter the mechanical efficiency of walking in pregnant women. Our final cohort consisted of a relatively homogenous group of women with respect to GWG and physical activity levels, and thus no prominent associations emerged. At an individual level, two relationships were apparent. The woman who experienced excessive GWG was the only woman to show a decrease in mechanical efficiency in late pregnancy. Similarly, the one woman who increased her activity in T3 was the only woman to exhibit an improved efficiency at this time point. However, these changes were minute (a difference of 1%), most likely representing methodological variations experienced over time, and not true changes in mechanical efficiency. The general patterns displayed between these variables in our cohort posits that GWG and physical activity levels have little effect on mechanical efficiency.

The direct linear relationship between body mass and W_{ext}, and between W_{ext} and energy expenditure suggests that it is unlikely that discordant GWG patterns would impact mechanical efficiency. In support of this, Davenport *et al.* found no change in the slope of work efficiency between pregnant obese and pregnant non-obese women in the second trimester (Davenport, Steinback, & Mottola, 2009). Additionally, load carriage more than 40 kg did not alter mechanical

efficiency in soldiers (Grenier et al., 2012). Similarly, we can postulate that physical activity habits would not alter mechanical efficiency. Early work by Margaria (1963) compared mechanical efficiency of running in athletes and non-athletes; he reported similar efficiencies between these populations. In contrast, recent work by McBride *et al.* (2015) looked at the mechanical efficiency of performing a repetitive jumping task and found that competitive runners performed the movement more efficiently than recreational runners. Mechanical efficiency has also been shown to increase for a given cycling task in women who underwent a 6-week long cycling endurance training program (Hintzy, Mourot, Perrey, & Tordi, 2005). A potential reason for the different conclusions could be related to the task performed. Even in those with limited experience or training, running is a relatively familiar task, whereas jumping and cycling are not as often performed in a sedentary lifestyle. Therefore a change in efficiency could be related to knowledge and experience with a given task. Research in children supports this suggestion. In childhood, the neuromuscular system is developing which contributes to the accrual of motor skills; as these motor skills are increasingly performed over time, the efficiencies of these movements improve. As such, compared to early childhood, children in late childhood display similar movement efficiencies to those seen in adults (Schepens, Bastien, Heglund, & Willems, 2004). Altogether, the fact that the exercise task applied in this thesis investigation was walking, further helps to support our findings that, in general, mechanical efficiency was unchanged and posits that it is unlikely a habitual activity level would greatly impact the mechanical efficiency of walking, a routine behaviour. However, if a more sport-specific task was to be evaluated, changes in efficiency may become apparent between novice and skilled pregnant women.

To our knowledge, the present thesis is the first to conclude that mechanical efficiency is unchanged across gestation. Previous examinations concluded that pregnant women had a superior

efficiency than what was displayed either in early pregnancy or non-pregnant states. There are a few methodological elements that can explain discrepancies in these findings. In the present examination, a treadmill task was performed that was standardized between all-time points and cohorts. Regardless of pregnancy state, participants were required to walk at a constant speed of 3.2 km/h for a total of 21-minutes. Every 3-minutes, the grade of the treadmill was increased in 2% increments, reaching a maximum incline of 14%. Seminal work by Margaria highlights the importance of keeping these variables consistent when exploring the efficiency of movement over time (Margaria, 1968). For walking, speed and efficiency relate in an inverted U pattern, whereas in running, they are linearly related. It is well established that pregnant women adopt a slower walking pace in late pregnancy (Branco et al., 2013; J. Davies et al., 2002), and therefore, to accurately assess changes efficiency during pregnancy, a standardized speed must be employed. Thus, the present work builds upon that of Lotgering *et al.* (1991) and Byrne *et al.* (2011) who assessed efficiency in pregnant women using self-selected walking speed. Another important consideration when characterizing mechanical efficiency during pregnancy is body mass changes. Increases in body mass are essential to optimal maternal-fetal health outcomes, but will undoubtedly impact energy dynamics and movement patterns. Several researchers have questioned the validity of presenting $\dot{V}O_2$ in relation to body mass, given that it misconstrues the accuracy of the value. For example, for a given exercise task, women with obesity will have a higher absolute $\dot{V}O2$ than their non-obese counterparts (because they are performing more absolute work); when scaled to body mass, the women with obesity have a lower $\dot{V}O_2$ (Hulens, Vansant, Lysens, Claessens, & Muls, 2001; Loftin et al., 2001). When looking at this in relation to efficiency, the biased reduction in $\dot{V}O_2$ results in the conclusion of greater mechanical efficiency; a finding that is grossly incorrect. The same principles apply in pregnancy; making values relative to body

weight underestimate the profound impact the higher body mass has on energy dynamics. In light of this, the current examination looked at variables only in absolute terms, which corrects for the oversight in the earlier work by Clapp (1989) and Byrne *et al.* (2011) which adjusted for body mass and concluded that efficiency was improved.

Our findings are in accordance with published literature addressing the change in mechanical efficiency in situations of external load carriage. We have shown that W_{ext} is directly related to GWG (Figure 6), but despite the change in energetics, mechanical efficiency is unaffected. Load carriage in soldiers has also displayed this relationship (Grenier et al., 2012; T. Huang & Kuo, 2014; Taylor et al., 2016). Although efficiency is not impacted by the change in GWG, one must consider the implications of higher workloads and energy demands for the same task. Pregnancy does not alter maximal aerobic capacity (Heenan et al., 2001; Lotgering et al., 1991) but a portion of a woman's energy reserves would be required to support and move the added load of the fetus and pregnancy-related tissues; this, in turn, increases the percentage of $\dot{V}O_{2max}$ at which she would be working for any given task. Of course, the metabolic cost of the task is increased, ultimately impacting the amount of work that is able to be performed, either in relation to the intensity or duration of the task. Moreover, the change in body mass is highly correlated to both energy expenditure and W_{ext} (Table 3). Therefore, it is important to understand these changes in the context of pregnancy to ensure guideline recommendations are not biased to those conclusions made from studies from non-pregnant populations. The women in our study required more energy to perform the walking task in late pregnancy than early pregnancy (Figure 3). On average, women experienced a 12% increase in energy expenditure at the T3 visit than at T1. Importantly, this highlights that physical activity will create a greater physiological challenge in pregnancy, but this increase – at least in the context of a moderate-intensity walking task – is

minimal and has little effect on the overall risk to mother and baby. This adds support to the growing body of evidence which debunks the longstanding myths about the safety concerns of being physically active in pregnancy (Szymanski & Satin, 2017). Further support for the safety of moderate exercise in pregnancy is the minimal change in HR observed to perform the task (Figure 7), and that the average HR_{max} attained during the task in late pregnancy did not exceed 150 bpm.

5.1 LIMITATIONS AND STRENGTHS

This study improved on the available literature within the context of mechanical efficiency and pregnancy. However, it still is constrained by a few methodological shortcomings. While a strength of the investigation was the addition of a well-matched non-pregnant comparator, the conclusions drawn may reflect individual differences in physiology instead of a true comparison between non-pregnant and pregnant states. The repeated measure design, whereby ten women were followed from early to late pregnancy, did allow for an accurate comparison of between early, mid- and late gestation. Secondly, the examination lacked a measurement of W_{int}, and so the results are also limited to what could be concluded based solely on W_{ext}. It might be of interest for future studies to consider W_{int} to gain comprehensive knowledge of the dynamics of whole-body efficiency over the course of pregnancy. To effectively measure internal work, prospective studies should measure the energy changes relative to the centre of mass (Cavagna & Kaneko, 1977; Willems et al., 1995), which would require biomechanical analysis, in addition to the measurement of the change in energy demand required to move the whole body relative to the ground (as we did in the current study). Nonetheless, a standardized treadmill task was used across all time points and between cohorts, which enabled a more accurate analysis of mechanical efficiency than the previously published literature. Notably, the chosen task is one that mimics a behaviour that is

encouraged in pregnant women to optimize health outcomes, and therefore the current findings add support to the promotion of physically active pursuits for all pregnant women.

The participant cohort may be criticized in two ways. Our participant sample size fell short of the estimates to achieve adequate power in our analyses to compare between the cohorts. Knowing that pregnancy lasts 40 weeks on average, a prospective study like this is time-consuming, and because the larger PLACENTA cohort accepted recruitment up until the 28th week, most women in the study were captured after the T1 time point. Fortunately, the sample recruited at T1 was great enough to support an analysis of adequate power for the pregnant cohort. The second criticism arises when looking at the homogeneity of our women. While it was hoped to recruit a diverse cohort of women, especially of varying pre-pregnancy BMI, GWG ranges or PA level, the final participant cohort included women who entered pregnancy at a healthy weight and gained weight within the current recommendations. The recruitment of a broader demographic could be sought in future studies. On an individual level, a relationship between GWG and mechanical efficiency, and between physical activity and mechanical efficiency were present in two women. These individual patterns suggest that a more heterogenous group may have led to different conclusions. However, given the overall findings in our cohort of an unchanged efficiency, and on the basis of fundamental physiological principles related to the metabolic cost of locomotion, it is postulated that it is unlikely that recruiting a wider demographic would produce different results than those obtained from the current investigation.

5.2 CONCLUSIONS

In conclusion, this thesis was the first to characterize mechanical efficiency across three-time points in pregnancy, and compare to non-pregnant controls, employing a methodology that

aligns with basic physiological concepts of energy dynamics and measurements of mechanical efficiency. This work, in which we characterized mechanical efficiency across gestation by exploring the relationship between energetic cost and W_{ext}, adds to the limited available literature related to exercise physiology in pregnancy. Previous concerns surrounding the ethical suitability of including pregnant women in research studies is slowly diminishing, as the reality that pregnant women are not as weak and vulnerable as once believed comes to light. Breaking down ethical barriers means saying goodbye to the days that pregnant women need not be considered in research; this ultimately helps to stop negative cycle of the past, whereby lack of evidence promotes false presumptions, leading to queries regarding the safety of including pregnant women in research (Foulkes, Grady, Spong, Bates, & Clayton, 2011).

In line with the results in other situations of load carriage, mechanical efficiency was unchanged in our pregnant cohort, despite the increase in energy requirements and external work. These findings suggest that increased body mass is the main contributor to increased energy expenditure during physical activity in pregnancy. Importantly, the conclusion that mechanical efficiency is not changed during pregnancy contributes to the growing body of evidence which demonstrates that responses to physical activity during pregnancy are not dissimilar to those seen in the non-pregnant state. From the prospective of mechanical efficiency, the work in this thesis illustrates that moderate physical activity can be performed in pregnancy without any undue stress on the body; this contributes to the body of knowledge which indicates that moderate physical activity is safe during pregnancy.

REFERENCES

2018 Physical Activity Guidelines for Americans. (2018). Washington, D.C.

ACOG. (1985). *Exercise during pregnancy and the postnatal period*.

BORN. (2016). *Better Outcomes Registry & Network (BORN) Ontario Annual Report 2014-2015 and 2015-2016*. Ottawa, ON.

Branco, M., Santos-Rocha, R., Aguiar, L., Vieira, F., & Veloso, A. (2013). Kinematic analysis of gait in the second and third trimesters of pregnancy. *Journal of Pregnancy*, *2013*. http://doi.org/10.1155/2013/718095

Brett, K. E., Ferraro, Z. M., Holcik, M., & Adamo, K. B. (2015). Prenatal physical activity and diet composition affect the expression of nutrient transporters and mTOR signaling molecules in the human placenta. *Placenta*, *36*(2), 204–212. http://doi.org/10.1016/j.placenta.2014.11.015

Brett, K. E., Wilson, S., Ferraro, Z. M., & Adamo, K. B. (2015). Self-report Pregnancy Physical Activity Questionnaire overestimates physical activity. *Canadian Journal of Public Health = Revue Canadienne de Sante Publique*, *106*(5), e297-302. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26451991

Butte, N., Hopkinson, J., Mehta, N., Moon, J., & O'Brian Smith, E. (1999). Adjustments in energy expenditure and substrate utilization during late pregnancy and lactation. *Am J Clin Nutr*, *69*, 299–307.

Butte, N., Wong, W. W., Treuth, M. S., Ellis, K. J., & Smith, B. (2004). Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am J Clin Nutr*, *79*, 1078–87.

Byrne, N. M., Groves, A. M., Mcintyre, D., & Callaway, L. K. (2011). Changes in resting and walking energy expenditure and walking speed during pregnancy in obese women. *Am J Clin Nutr*, *94*, 819–30. http://doi.org/10.3945/ajcn.110.009399

Carpenter, M. W., Sady, S. P., Sady, M. A., Haydon, B., Coustan, D. R., & Thompson, P. D. (1990). Effect of maternal weight gain during pregnancy on exercise performance. *Journal of Applied Physiology*, *68*(3), 1173–1176.

Cavagna, G., & Kaneko, M. (1977). Mechanical work and efficiency in level walking and running. *J Physiol*, *268*, 467–481.

Cavagna, G., Saibene, F. P., & Margaria, R. (1963). External work in walking. *Journal of Applied Physiology*, *18*(3), 1–9. http://doi.org/10.1152/jappl.1963.18.1.1

Cavanagh, P. R., & Kram, R. (1985). Mechanical and muscular factors affecting the efficiency of human movement. *Medicine and Science in Sports and Exercise*. http://doi.org/10.1249/00005768-198506000-00005

Clapp, J. (1989). Oxygen consumption during treadmill exercise before, during, and after pregnancy. *American Journal of Obstetrics and Gynecology*, *161*, 1458–1464. http://doi.org/10.1016/0002-9378(89)90904-6

CSEP. (2013). *Canadian Society for Exercise Physiology - Physical Activity Training for Health (CSEP-PATH) Resource Manual*. Ottawa, ON.

Colley, R. (2012). Actical accelerometer data analysis support tool: harmonizing with the Canadian Health Measures Suryve. *ACCEL+ Version 1.0 User Guide.*

Davenport, M. H., Steinback, C. D., & Mottola, M. F. (2009). Impact of pregnancy and obesity on cardiorespiratory responses during weight-bearing exercise. *Respiratory Physiology & Neurobiology*, *167*, 341–347. http://doi.org/10.1016/j.resp.2009.06.009

Davies, G., Wolfe, L. A., Mottola, M. F., & MacKinnon, C. F. (2003). Exercise in pregnancy and the postpartum period. *Journal of Obstetrics and Gynaecology Canada*, *25*(6), 516–522.

Davies, J., Fernando, R., McLeod, A., Verma, S., & Found, P. (2002). Postural stability following ambulatory regional analgesia for labor. *Anesthesiology*, *97*(6), 1576–81. http://doi.org/10.1097/00000542-200212000-00033

Denize, K., Acharya, N., Ferraro, Z., Harvey, A., S, P. W., & KB, A. (2018). The effects of culture on guideline discordant gestational weight gain: A systematic review and meta-analysis. *PeerJ*, *In Press.*

Downs, D. S., Chasan-Taber, L., Evenson, K. R., Leiferman, J., & Yeo, S. (2012). Physical Activity and Pregnancy: Past and Present Evidence and Future Recommendations. *Res Q Exerc Sport*, *83*(4), 485–502.

Dunning, K., LeMasters, G., Levin, L., Bhattacharya, A., Alterman, T., & Lordo, K. (2003). Falls in workers during pregnancy: risk factors, job hazards, and high risk occupations. *Am J Ind Med*, *44*, 664–672.

Durnin, J. (1991). Energy requirements of pregnancy. *Diabetes1*, *40*(Supplement 2), 152–156.

Federal Security Agency and Social Security Administration. (1949). *Prenatal care*. Children's Bureau Publication.

Fenn, W. (1930). Frictional and kinetic factors in the work of sprint running. *Am J Physiology*, *93*, 483–611.

Ferraro, Z. M., Gaudet, L., & Adamo, K. B. (2012a). The potential impact of physical activity during pregnancy on maternal and neonatal outcomes. *Obstetrical & Gynecological Survey*, *67*(2), 99–110. http://doi.org/10.1097/OGX.0b013e318242030e

Ferraro, Z. M., Gaudet, L., & Adamo, K. B. (2012b). The Potential Impact of Physical Activity During Pregnancy on Maternal and Neonatal Outcomes. *Obstetrical & Gynecological Survey*, *67*(2), 99–110. http://doi.org/10.1097/OGX.0b013e318242030e

Forczek, W., & Staszkiewicz, R. (2012). Changes of kinematic gait parameters due to pregnancy. *Acta of Bioengineering and Biomechanics Original Paper*, *14*(4). http://doi.org/10.5277/abb120413

Foti, T., Davids, J. R., & Bagley, A. (2000). A biomechanical analysis of gait during pregnancy. *The Journal of Bone and Joint Surgery*, *82*(5), 625–632.

Foulkes, M. A., Grady, C., Spong, C. Y., Bates, A., & Clayton, J. A. (2011). Clinical Research Enrolling Pregnant Women: A Workshop Summary. *Journal of Women's Health*, *20*(10), 1429–1432. http://doi.org/10.1089/jwh.2011.3118

Gaston, A., & Cramp, A. (2011). Exercise during pregnancy: A review of patterns and determinants. *Journal of Science and Medicine in Sport*, *14*, 299–305. http://doi.org/10.1016/j.jsams.2011.02.006

Gilmore, L. A., Butte, N. F., Ravussin, E., Han, H., Burton, J. H., & Redman, L. M. (2016). Energy intake and energy expenditure for determining excess weight gain in pregnant women. *Obstet Gynecol*, *127*(5), 884–892.

Girling, J. C. (2004). Physiology of Pregnancy. *Anaesthesia and Intensive Care Medicine*, *5*(7), 215–218. http://doi.org/10.1136/bmj.1.5426.44

Granger, J. P. (2002). Maternal and fetal adaptations during pregnancy: lessons in regulatory and integrative physiology. *Am J Physiol Regul Integr Comp Physiol*, *283*, R1289–R1292. http://doi.org/10.1152/ajpregu.00562.2002

Grenier, J. G., Peyrot, N., Castells, J., Oullion, R., Messonnier, L., & Morin, J. B. (2012). Energy cost and mechanical work of walking during load carriage in soldiers. *Medicine and Science*

in Sports and Exercise, *44*(6), 1131–1140. http://doi.org/10.1249/MSS.0b013e3182456057
Guelfi, K. J., Wang, C., Dimmock, J. A., Jackson, B., Newnham, J. P., & Yang, H. (2015). A comparison of beliefs about exercise during pregnancy between Chinese and Australian pregnant women. *BMC Pregnancy and Childbirth*, *15*(345). http://doi.org/10.1186/s12884-015-0734-6
Hagan, L., & Wong, C. . (2010). Gait in pregnant women: spinal and lower extremity changes form pre- to postpartum. *Research Report*, *34*(2).
Heenan, A. P., Wolfe, L. A., & Davies, G. A. L. (2001). Maximal exercise testing in late gestation: maternal response. *Obstetrics & Gynecology*, *97*(1), 127–134.
Hinman, S. K., Smith, K. B., Quillen, D. M., & Smith, M. S. (2015). Exercise in Pregnancy. *Sports Health: A Multidisciplinary Approach*, *7*(6), 527–531. http://doi.org/10.1177/1941738115599358
Hintzy, F., Mourot, L., Perrey, S., & Tordi, N. (2005). Effect of endurance training on different mechanical efficiency indices during submaximal cycling in subjects unaccustomed to cycling. *Canadian Journal of Applied Physiology*, *30*(5), 520–8.
Huang, L., Chen, P., Zhuang, J., Zhang, Y., & Walt, S. (2013). Metabolic cost, mechanical work, and efficiency during normal walking in obese and normal-weight children. *Research Quarterly for Exercise and Sport*, *84*, S72–S79. http://doi.org/10.1080/02701367.2013.849159
Huang, T., & Kuo, A. (2014). Mechanics and energetics of load carriage during human walking. *Journal of Experimental Biology*, *217*(4), 605–613. http://doi.org/10.1242/jeb.091587
Hulens, M., Vansant, G., Lysens, R., Claessens, A. L., & Muls, E. (2001). Exercise capacity in lean versus obese women. *Scandinavian Journal of Medicine & Science in Sports*, *11*(5), 305–309. http://doi.org/10.1034/j.1600-0838.2001.110509.x
Huppertz, B., & Peeters, L. L. H. (2005). Vascular biology in implantation and placentation. *Angiogenesis*, *8*, 157–167. http://doi.org/10.1007/s10456-005-9007-8
Jang, J., Hsiao, K. T., & Hsiao-Wecksler, E. T. (2008). Balance (perceived and actual) and preferred stance width during pregnancy. *Clinical Biomechanics*, *23*(4), 468–476. http://doi.org/10.1016/j.clinbiomech.2007.11.011
Knöfler, M. (2010). Critical growth factors and signalling pathways controlling human trophoblast invasion. *The International Journal of Developmental Biology*, *54*(2–3), 269–80. http://doi.org/10.1387/ijdb.082769mk
Knuttgen, H., & Emerson, K. (1974). Physiological response to pregnancy at rest and during exercise. *Journal of Applied Physiology*, *36*(5).
Lafortuna, C., Agosti, F., Galli, R., Busti, C., Lazzer, S., & Sartorio, A. (2008). The energetic and cardiovascular response to treadmill walking and cycle ergometer exercise in obese women. *Eur J Appl Physiol*, *103*, 707–717.
Lof, M., Olausson, H., Bostrom, K., Janerot-Sjöberg, B., Sohlstrom, A., & Forsum, E. (2005). Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin- like growth factor I, and thyroid hormones and in relation to fetal growth. *The American Journal of Clinical Nutrition*, *81*, 678–685.
Loftin, M., Sothern, M., Trosclair, L., O'Hanlon, A., Miller, J., & Udall, J. (2001). Scaling VO2 peak in obese and non-obese girls. *Obesity Research*, *9*(5), 290–6. http://doi.org/10.1038/oby.2001.36
Lotgering, F. K., Van Doorn, M. B., Struijk, P. C., Pool, J., & Wallenburg, H. C. S. (1991). Maximal aerobic exercise in pregnant women: heart rate, O2 consumption, CO2 production,

and ventilation. *J Appl Physiol*, *70*(3), 1016–1023.
Maeder, E. C. (1977). Effects of sport and exercise in pregnancy. *Postgraduate Medicine*, *2*, 112–114.
Margaria, R. (1968). Positive and negative work performances and their efficiencies in human locomotion. *Internationale Zeitschrift Für Angewandte Physiologie Einschließlich Arbeitsphysiologie*, *25*(4), 339–351. http://doi.org/10.1007/BF00699624
Margaria, R., Cerretelli, P., Aghemo, P., Sass1, G., & Sassi, G. (1963). Energy cost of running. *J Appl Physiol*, *18*(2), 367–370.
McAuliffe, F., Kametas, N., Costello, J., Rafferty, G. F., Greenough, A., & Nicolaides, K. (2002). Respiratory function in singleton and twin pregnancy. *BJOG: An International Journal of Obstetrics and Gynaecology*, *109*(7), 765–769. http://doi.org/10.1111/j.1471-0528.2002.01515.x
McBride, J. M., Davis, J. A., Alley, J. R., Knorr, D. P., Goodman, C. L., Snyder, J. G., & Battista, R. A. (2015). Index of mechanical efficiency in competitive and recreational long distance runners. *Journal of Sports Sciences*, *33*(13), 1388–1395. http://doi.org/10.1080/02640414.2014.990487
McCrory, J., Chambers, A., Daftary, A., & Redfern, M. (2010). Dynamic postural stability during advancing pregnancy. *Journal of Biomechanics*, *43*, 2434–2439. http://doi.org/10.1016/j.biomech.2009.09.058
Melzer, K., Schutz, Y., Soehnchen, N., Othenin Girard, V., Martinez de Tejada, B., Pichard, C., … Kayser, B. (2010). Prepregnancy body mass index and resting metabolic rate during pregnancy. *Annals of Nutrition & Metabolism*, *57*(3–4), 221–7. http://doi.org/10.1159/000322369
Most, J., Vallo, P. M., Gilmore, L. A., St. Amant, M., Hsia, D. S., Altazan, A. D., … Redman, L. M. (2018). Energy Expenditure in Pregnant Women with Obesity Does Not Support Energy Intake Recommendations. *Obesity*, *26*(6), 992–999. http://doi.org/10.1002/oby.22194
Moya, J., Phillips, L., Sanford, J., Wooton, M., Gregg, A., & Schuda, L. (2014). A review of physiological and behavioral changes during pregnancy and lactation: Potential exposure factors and data gaps. *Journal of Exposure Science and Environmental Epidemiology*, *24*(10), 449–458. http://doi.org/10.1038/jes.2013.92
Nascimento, S. L., Surita, F. G., & Cecatti, J. G. (2012). Physical exercise during pregnancy. *Current Opinion in Obstetrics and Gynecology*, *24*(6), 387–394. http://doi.org/10.1097/GCO.0b013e328359f131
O'Toole, M. L. (2003). Physiologic aspects of exercise in pregnancy. *Clinical Obstetrics and Gynecology*, *46*(2), 379–389. http://doi.org/10.1097/00003081-200306000-00017
Pedotti, A. (1977). A Study of Motor Coordination and Neuromuscular Activities in Human Locomotion. *Biol. Cybernetics*, *26*, 53–62.
Pernoll, M. L., Metcalfe, J., Schlenker, T. L., Welch, J. E., & Matsumoto, J. A. (1975). Oxygen consumption at rest and during exercise in pregnancy. *Respiration Physiology*, *25*(3), 285–93.
Robson, S. C., Hunter, S., Boys, R. J., & Dunlop, W. (1988). Serial study of factors influencing changes in cardiac output during human pregnancy. *American Journal of Physiology - Heart and Circulatory Physiology*, *256*(25), HI060-H1065.
Saibene, F., & Minetti, A. (2003). Biomechanical and physiological aspects of legged locomotion in humans. *Eur J Appl Physiol*, *88*, 297–316. http://doi.org/10.1007/s00421-002-0654-9

San-Frutos, L., Engels, V., Zapardiel, I., & et al. (2011). Haemodynamic changes during pregnancy and postpartum: a prospective study using thoracic electrical bioimpedance. *The Journal of Maternal-Fetal & Neonatal Medicine.*, *24*(11), 1333–40.

Sattler, M. C., Jaunig, J., Watson, E. D., Van Poppel, M. N. M., Mokkink, L. B., Terwee, C. B., … Sattler, M. C. (2018). Physical activity questionnaires for pregnancy: A systematic review of measurement properties. *Sports Medicine*. http://doi.org/10.1007/s40279-018-0961-x

Schepens, B., Bastien, G., Heglund, N., & Willems, P. (2004). Mechanical work and muscular efficiency in walking children. *The Journal of Experimental Biology*, *207*, 587–596. http://doi.org/10.1242/jeb.00793

Simonsen, E., & Alkjaer, T. (2012). The variability problem of normal human walking. *Medical Engineering & Physics*, *34*, 219–224.

Snyder, J. L. (1990). Aerobic Exercise During Pregnancy. *J Am Bd Fm Pract*, *3*, 50–53.

Symons Downs, D., & Hausenblas, H. A. (2004). Women's Exercise Beliefs and Behaviors During Their Pregnancy and Postpartum. *J Midwifery Womens Health*, *49*, 138–144. http://doi.org/10.1016/j.jmwh.2003.11.009

Szymanski, L. M., & Satin, A. J. (2017). Exercise During Pregnancy. *JAMA*, *317*(11), 1113–1114.

Tan, E., & Tan, E. (2013). Alterations in physiology and anatomy during pregnancy. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *27*(6), 791–802. http://doi.org/10.1016/j.bpobgyn.2013.08.001

Taylor, N. A. S., Peoples, G. E., & Petersen, S. R. (2016). Load carriage, human performance, and employment standards. *Appl Physiol Nutr Metab*, *41*, S131–S147. http://doi.org/10.1139/apnm-2015-0486

Willems, P. A., Cavagna, G. A., Heglund, N. C., Umana, F., & Chelmsford, S. (1995). External, internal and total work in human locomotion, *393*, 379–393.

Wolfe, L. A., & Mottola, M. F. (1993). Aerobic exercise in pregnancy: an update. *Canadian Journal of Applied Physiology* , *18*(2), 119–47.

APPENDIX A: ETHICAL APPROVALS

Ottawa Hospital Research Institute
Institut de recherche de l'Hôpital d'Ottawa

uOttawa

University of Ottawa Heart Institute
Institut de cardiologie de l'Université d'Ottawa

Ottawa Health Science Network Research Ethics Board/ Conseil d'éthique de la recherche du Réseau de science de la santé d'Ottawa
Civic Box 411 725 Parkdale Avenue, Ottawa, Ontario K1Y 4E9 613-798-5555 ext. 14902 Fax : 613-761-4311
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June 5, 2018

Dr. Laura Gaudet

Dear Dr. Gaudet:

RE: Protocol# - 20160178-01H **PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA)**

Renewal Expiry Date - June 4, 2019

I am pleased to inform you that your Annual Renewal Request was reviewed by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) and is approved. No changes, amendments or addenda may be made in the protocol or the consent form without the OHSN-REB's review and approval.

Date of renewal approval: June 5, 2018

Rose Vuong has been added as staff.

If the study is to continue beyond the expiry date, a Renewal Form should be submitted to the REB, in hardcopy. All Annual Renewal Reports, regardless of review type (i.e., full board or delegated), must now be submitted according to the full board meeting submission deadlines AND at least 30 days prior to the expiry date of the study to prevent a lapse in approval. If the study is completed by this date, a Termination Report should be submitted.

The consent forms currently approved for use by the REB are:
- English Participant Informed Consent Form dated November 13, 2017
- French Participant Informed Consent Form dated November 13, 2017

The OHSN-REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline and the provisions of the Personal Health Information Protection Act 2004.

Yours sincerely,

Raphaël Saginur, M.D.
Chairperson
Ottawa Health Science Network Research Ethics Board

/kd

Montfort
Hôpital universitaire
Academic Hospital

Avis de renouvellement de l'approbation éthique
Comité d'éthique de la recherche (CÉR) de l'Hôpital Montfort

Le 14 août 2017

Chercheures principales :

Dr Laura Gaudet

Kristi Adamo, Ph. D

Collaboratrice et responsable de site :

Dre Natalie Gauthier

Titre du projet: « **P**hysica**L** **AC**tivity and di**E**tary implicatio**NsT**hroughout pregn**A**ncy (PLACENTA) »

Numéro du dossier : LG-01-06-16

Date de début : 30 août 2017

Date de fin : 29 août 2018

Le Bureau d'éthique de la recherche (BÉR) de l'Hôpital Montfort vous informe que votre demande de renouvellement annuel de l'approbation éthique pour le projet mentionné ci-dessus a été **évaluée** et **approuvée** par le président du CÉR ou son délégué. Les décisions prises au sujet des dossiers évalués en comité délégué sont ratifiées par le comité lors de sa prochaine réunion plénière.

Le protocole de l'étude ne peut être modifié sans une approbation préalable du CÉR sauf s'il est question de la sécurité immédiate des participants. Le chercheur doit, avant toute utilisation, soumettre pour évaluation et approbation toutes les modifications au protocole et à la documentation destinée aux participants, par exemple, formulaire de consentement et aux outils de recrutement. Vous devez aussi aviser le CÉR immédiatement de tout événement indésirable ou nouvelle information pouvant augmenter le risque ou modifier le cours du projet de recherche.

Votre certificat d'approbation est valide pour les dates de début et de fin mentionnées ci-dessus et vous devriez nous acheminer **quatre semaines avant la date d'échéance de cet avis d'approbation**, un rapport final ou d'étape annuel afin de fermer le dossier ou de faire une demande de renouvellement de l'approbation éthique. Veuillez noter que vous pouvez soumettre votre demande de renouvellement plus tôt que le délai exigé. Toutefois, le CÉR évaluera et émettra le certificat de renouvellement à une date qui ne remonte pas à plus tôt que 30 jours avant la date d'expiration du certificat valide.

Le CÉR de l'Hôpital Montfort est constitué et exerce ses activités d'une manière conforme à la Norme nationale du Canada visant la surveillance de l'éthique de recherches comportant des essais cliniques biomédicaux de l'Office des normes générales du Canada, aux

713 Montréal, Ottawa, ON K1K 0T2
613-746-4621
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www.hopitalmontfort.com

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Kristi	Adamo	Health Sciences / Human Kinetics	Principal Investigator
John Thor	Arnason	Science / Biology	Co-investigator
Shannon	Bainbridge	Health Sciences / Others	Co-investigator
Zach	Ferraro	Health Sciences / Human Kinetics	Co-investigator
Laura	Gaudet	Medicine / Medicine	Co-investigator
Martin	Holcik		Co-investigator
Jameason	Cameron	Health Sciences / Human Kinetics	Other Collaborator
Nathalie	Gauthier		Other Collaborator
David	Grynspan	Medicine / Medicine	Other Collaborator
Alysha	Harvey	Health Sciences / Human Kinetics	Project Coordinator

File Number: H11-15-29

Type of Project: Professor

Title: Physical activity and dietary implications throughout pregnancy (PLACENTA)

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
09/01/2017	08/31/2018	Renewal

550, rue Cumberland, pièce 154 550 Cumberland Street, room 154
Ottawa (Ontario) K1N 6N5 Canada Ottawa, Ontario K1N 6N5 Canada
(613) 562-5387 • Téléc./Fax (613) 562-5338
www.recherche.uottawa.ca/deontologie/ www.research.uottawa.ca/ethics/

Queensway Carleton Hospital

September 13th, 2017

Dr. Kristi Adamo

RE: Study 17-03: PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA)

Protocol approval valid until: September 30th, 2018

Dear Dr. Adamo:

Thank you for your submission of the application for approval regarding the above named study. I am pleased to inform you that your study has been approved, and this will extend to September 30th, 2018. We request that two months prior to that date you submit an annual renewal form, at which time you may request an extension of approval if so indicated. We also require an end-of-study report to be submitted as soon as possible from the date of completion or termination of your research, by submitting a termination form. Please note that you are required to request approval from the QCH REB for any and all modifications by which you plan to amend your protocol. Copies of all forms can be accessed on our website at www.qch.on.ca/REB.

The REB requires Investigators to comply with the guidelines described in the Tri-Council Policy Statement (2014): Ethical Conduct for Research Involving Humans –TCPS-2, as well as other regulatory standards for research practice in Ontario/Canada which are pertinent to your study.

Thank you and should you have any questions, please do not hesitate to contact me.

Sincerely,

B. Gannon, MD, MSc., FRCPC
Chair, QCH REB

cc Dr. Andrew Falconer, Chief of Staff
Ms. Alysha Harvey, Clinical Research Associate
Ms. Yvonne Zinck, Manager, Health Records

RESEARCH INSTITUTE
INSTITUT DE RECHERCHE

Research Ethics Board
2018 Annual Renewal (Delegated)

Principal Investigator: Dr. David Grynspan

REB Protocol No: 16/68X
Romeo File No: 20160264
Project Title: CHEOREB# 16/68X - PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA)
Primary Affiliation: Clinical Research\Pathology
Protocol Status: Active
Approval Date: May 7, 2018
Approval Valid Until: June 15, 2019
Annual Renewal Submission Deadline: May 15, 2019

This is to notify you that the CHEO REB has granted approval to the renewal for the above named research study for a period of one year. The renewal was reviewed and approved by the Chair or a delegate of the Chair. Decisions made by the Chair under delegated review are ratified by the full Board at its subsequent meeting.

Approval is granted with the understanding that the investigator agrees to comply with the following requirements:

1. The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.
2. The investigator is responsible for complying with all applicable guidelines and regulations regarding human research ethics conduct, as applicable to the research project.
3. Investigators must submit an annual renewal report to the REB 30 days pror to the expiration date stated on the final approval letter.
4. The investigator must not implement any deviation from, or changes to, the protocol without the approval of the REB except where necessary to eliminate an immediate hazard to the research subject, or when the change involves only logistical or administrative aspects of the study (e.g., change of

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

H-11-17-190 - REG-190 - Certificat d'approbation éthique / Certificate of Ethics Approval

(Please scroll down for the English version)

Cher/Chère Kathryn Denize,

Veuillez trouver le certificat d'approbation éthique pour le projet intitulé : Characterization of mechanical efficiency and thermal strain in healthy adult women. .

Le certificat est valide jusqu'au : 29-11-2018

Si vous avez reçu une subvention pour le projet de recherche, veuillez faire suivre une copie du certificat d'approbation éthique au Service de gestion de la recherche à http://research.uottawa.ca/rms/about.

Si vous avez des questions, n'hésitez pas à communiquer avec le bureau d'éthique à ethique@uottawa.ca ou en composant le 613-562-5387.

Vous pouvez voir votre demande en vous connectant à votre compte eReviews.

Très cordialement,

Riana Marcotte

Responsable d'éthique en recherche

Ceci est une réponse automatisée, merci de ne pas répondre à ce courriel.

Dear Kathryn Denize,

Please find attached the certificate of ethics approval for your research project entitled: "Characterization of mechanical efficiency and thermal strain in healthy adult women. ".

This certificate is valid until: 29-11-2018

A reminder that if you received a grant for this research, you must provide a copy of your ethics certificate to Research Management Services at http://research.uottawa.ca/rms/about.

If you have any questions, please contact the Ethics Office at ethique@uottawa.ca or by telephone at 613-562-5387.

You can view your project at any time by logging into eReviews.

Best regards,

Riana Marcotte

Protocol Officer

This is an automated message. Please do not reply directly to this email.

Attachement(s) / Attachment(s)
approvalLetter1512063535824.pdf

550, rue Cumberland, pièce 154 550 Cumberland Street, Room 154
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www.recherche.uottawa.ca/deontologie | www.recherche.uottawa.ca/ethics

APPENDIX B: INFORMED CONSENT FORM (PLACENTA)

PARTICIPANT INFORMED CONSENT FORM

Title of Study: Physica**L AC**tivity and di**E**tary implicatio**N**s **T**hroughout pregn**A**ncy **(PLACENTA)**

Investigators:

Kristi Adamo, Ph.D.	Associate Professor, University of Ottawa 613-562-5800 x 1009
Dr. Laura Gaudet	Associate Scientist, Ottawa Hospital Research Institute 613-737-8899 x 76655
Martin Holcik, Ph.D.	CHEO Research Institute
David Grynspan, Ph.D.	CHEO Pathology
Shannon Bainbridge, Ph.D.	Ottawa Hospital Research Institute University of Ottawa
Jane Shearer, Ph.D.	University of Calgary
John T. Arnason, Ph.D.	University of Ottawa
Zach Ferraro, Ph.D.	University of Ottawa

Collaborators:

Dr. Natalie Gauthier	Hôpital Montfort
Jameason Cameron, Ph.D.	CHEO Research Institute
Dr. Liisa Honey	Queensway Carleton Hospital
Research Coordinator:	University of Ottawa 613-562-5800 x1012

Funding Agency: Canadian Institute of Health Research (CIHR)

Participation in this study is voluntary. Please read this Participant **Informed** Consent Form carefully before you decide if you would like to participate. Please ask the principal investigator and the study team as many questions as you like. We encourage you to discuss your options with family, friends or your health care provider.

Why am I being given this form?

You are being asked to participate in this research study because you are pregnant and planning to deliver locally and have indicated that you are interested in learning more about our research study.

Why is this study being done?

The PLACENTA study is designed to look at how lifestyle behaviours during pregnancy can affect how nutrients move to the growing fetus. The placenta is an organ that forms during pregnancy to support the baby. The placenta controls the transfer of nutrients (sugar, protein and fat) and oxygen to the fetus, and removes waste. If the placenta is not working properly, the baby may receive too few or too many nutrients. It is not well known how health behaviours, like exercise or nutrition, affect the transfer of nutrients to the fetus.

The goal of this study is to see if there are differences in the placenta's ability to transfer nutrients in pregnant women who exercise regularly compared to women who do not exercise regularly. This study is an observational study, we do not want you to make changes to your lifestyle because you are part of this study. We are simply looking to observe the natural differences between people and the impact that those differences may have.

During pregnancy, the placenta plays an important role in the growth and/or overgrowth of the baby. Based on work done by our team and others we believe that healthy eating and physical activity will be linked to differences in:

A) The way that new blood vessels form from existing ones (in the way that your cells respond to oxygen)
B) In the way that nutrients and oxygen move across the placenta (both of which are key controllers of the baby's growth).

This study will compare samples of the placenta from women who were physically active throughout their pregnancy and those who were not. We will explore the impact of physical activity on the genes that control the transfer of nutrients across the placenta to the fetus. We will also analyze the nutritional, environmental, and genetic factors that may affect placenta function by studying the relationship between genes/DNA and natural processes that occur during pregnancy which determine how a baby grows while in the womb and after birth. Genes are the building blocks or instructions found inside cells, which contribute to the different features you have, such as the colour of your hair and eyes. We hope that the information we learn in this study will be helpful in designing preventive health strategies for pregnancy.

How is the study designed? What is expected of me?

If you consent to participate in this study, you will be asked to come to the University of Ottawa-Lees Campus to meet our research team once during each trimester at specific times and dates which will be arranged between you and the study's research coordinator. Our goal

is to recruit a group of women in their first trimester of pregnancy and should you attend your first visit during your first trimester, you will attend three (3) visits in total. However, many women do not know that they are pregnant or see their doctor before their second trimester. If you are recruited in your second trimester, you will be asked to come for two (2) visits.

The first trimester visit will occur between weeks 12-16 of your pregnancy. The second trimester visit will occur between weeks 24-28 of your pregnancy. The third and final visit will occur between weeks 34-38 of your pregnancy. The estimated length of each visit is 1.5 hours. Visits will take place during the week or on the weekend at a time that is convenient for you.

Visit details: first, second and third trimester

These visits will take approximately 1.5 hours each.

1) A small fasting (no food for 8 hours before) blood sample (2 teaspoons or 10 ml) will be taken. It is best if we book your appointments for first thing in the morning, before you have eaten breakfast. This blood sample will be used to measure metabolic markers associated with nutrient transport, fetal growth, and weight gain.

2) After the blood sample, you be given a small snack, for example a granola bar and some juice (or you can bring your own snack).

3) You will be asked to complete a few questionnaires:
    a. Socio-demographic information (first visit only)
    b. General health behaviours
    c. Diet History Questionnaire (first visit only)
    d. Edinburgh Postnatal Depression Score

4) We will measure your height and weight.

5) We will then ask you to put on a heart rate monitor, and skin temperature sensor stickers and rest seated for 20 minutes in order to collect your resting energy requirements and heart rate. After the period of seated rest, we will ask you to walk on a treadmill at a comfortable pace (2 mph, 3.2 kph). The incline of the treadmill will increase every 3 minutes for approximately 20-25 minutes, but you can choose to stop at any point. At the end of each stage, we will ask you how hard you feel that you are working (Borg scale of perceived exertion), how hot you feel (thermal sensation scale), and we will measure your body temperature using an ear thermometer. We will measure your heart rate and stop the test before you reach 85% of their maximum estimated heart rate.

6) You will be asked to complete three 24-hour dietary recalls (food and drinks) in the week following each visit (2 week days, 1 weekend day), this will take about 20

minutes/day. You can do this online with information that we will give you, or on paper if you don't have access to the internet.

7) You will be given a small physical activity monitor to wear for 1-week after each visit. It is a 'smart' pedometer that works like the motion sensor (counting steps) but also provides information on your speed and direction of movement. It is always on but it is activated only by movement. This will give us an idea of your typical physical activity habits. The monitor (1" square) is safe, relatively small, non-invasive and is worn on a soft belt around the waist either under or over clothing and will not impact day-to-day activities. While we hope that you treat your accelerometer responsibly, please note that in the case of loss or breakage of the physical activity monitor, you will not be responsible for costs of replacement.

8) In the week following the visit, you will be asked to collect a small stool sample using a collection kit that we will provide you. You will then mail the kit, the physical activity monitor, and the physical activity log sheet to our office in the pre-addressed, pre-stamped envelope that we will provide you.

Visit details: At birth and 24-48 hours post-birth

This visit will take approximately 10 minutes.

1) When you arrive at the hospital/birthing unit in labour, please mention that you are part of our study. We will already have given the hospital/birthing unit a note in your file (and a door magnet) to indicate that you are participating in our study and to remind the hospital/birthing unit staff to call the research team for placenta sampling if you present at or greater than 37 weeks gestation. If you arrive in labour less than 37 weeks gestation, you will be excluded from the placenta sampling and infant measurements. The note will include your name, date of birth, the phone number to reach our research team, and brief sample processing directions for blood samples and placenta and will be kept in your paper file but not in your electronic online hospital file. We will not interact with you while you are in labour. The Labour and Delivery clerk or Midwife will call the research team to visit the hospital/birthing unit/home to pick up your placenta for sampling.

2) Once the placenta is no longer attached to you or your baby, the nursing staff or midwife will provide us with your placenta so that we can take a blood sample (5 –10mL or 1 - 2 teaspoons) from the umbilical cord, weigh the placenta and take tissue samples from it. This will not involve any pain and will not involve sticking your baby with a needle. These samples will be used to assess markers associated with nutrient transport, growth and development. Our sampling may prevent you from taking part in

a private cord blood storage program. The blood sample that we store for research purposes will not be available to you for any other purposes.

3) We will return to the hospital 24 – 48 hours after you have your baby, to measure the infant's height, weight, and body composition (skin folds). If you have been released by the hospital by this time, we will visit your home for the very short assessment. We will confirm these details with you during your last visit at the Lees Campus.

4) Approximately one (1) week after you give birth, you will be asked to collect a small stool sample from yourself, as well as one from your baby, and place them into the special collection kits that we will have given to you. We will call you to remind you to send in your samples. You will then mail the kit to our office in the pre-addressed, pre-stamped envelope that we will provide you.

5) Access to your medical charts from your delivery will be made through the service of clinical archives of the Montfort hospital or the Ottawa Hospital to obtain information on a variety of measures that are conducted and recorded as part of your standard care unrelated to the study. We will collect information such as your child's sex, date of birth, birth weight, crown-heel length, if your baby was born premature or at term, and APGAR scores. We will also retrieve information about you, including your age, height, weight and gestational age at the time of delivery, index of parity, the weight of your placenta, level of labour pain, method of delivery, recorded alcohol and drug consumption during pregnancy, OGTT scores, pregravid weight, and any complications that arose during pregnancy or delivery.

Table 1: Study Visits and Procedures

Boxes marked with an X show what will happen at each visit.

Visit	**1st trimester visit (weeks 12-16) Sub-sample early recruits only**	**2nd trimester visit (weeks 24-28) ALL participants**	**3rd trimester visit (weeks 34-38) ALL participants**	**Birth & Post Birth**
Length of time needed	1.5 hours	1.5 hours	1.5 hours	10 minutes with baby
Maternal height and weight	X	X	X	
Questionnaires	X	X	X	
Blood Sample	X	X	X	
Fitness Test	X	X	X	
Placenta processing				X (birth)
Infant length, weight and body composition				X (24 hrs post-birth)
Stool samples (Maternal all visits and post-birth) & Baby post-birth	X	X	X	X (1 week post-birth) X (baby)

If you are interested, you can choose to allow us to contact you for future research studies done by our study team related to health behaviours and pregnancy outcomes. This is not a mandatory part of your participation for this study. The signature page at the end of this document allows you to indicate your preference. Also, you will be asked to sign a separate consent form for any future research projects that you may optionally be contacted about and wish to participate in.

Will my samples be used in future research studies?

If you accept this optional component, any leftover blood/DNA and placenta tissue as well as leftover samples of your baby's blood/DNA will be stored for future research studies related to placenta function. The study of biology is rapidly evolving, as is our understanding of genetics and more notably something called 'epigenetics'. This is a relatively new area of research and simply put, epigenetics is the study of biological mechanisms that can switch genes on and off. For example, what you eat, where you live, who you interact with, when you sleep, how you exercise, and even aging can change the way our genes are turned on or off over time. Some of these changes can be inherited and the different combinations of genes that are turned on or off are what makes each one of us unique. We would like to have the opportunity to examine

this further as it relates to behaviours in pregnancy. Research Ethics Board approval will be sought before any future research begins.

We will take the baby's sample from the umbilical cord once it is cut away from the baby after delivery and no longer attached to you or your baby. Similarly, the placenta tissue sample will be taken after it has been delivered and thus will not involve any pain whatsoever nor will these procedures involve sticking your baby with a needle.

All samples will be coded (they will not contain any of your personal identifying information) and will be kept in a locked –80°C freezer located Kristi Adamo's lab at the University of Ottawa-Lees Campus for an indeterminate period of time. All of your personal health information collected will be de-identified and associated with only a study ID number, however a password protected master list containing your name and study ID number will exist and be accessible only to the Clinical Research Coordinator conducting the study.

As recommended, all the samples will be owned and governed by the Principal Investigator, Kristi Adamo, Ph.D. In order to protect the biospecimen integrity, the samples will be kept in a locked –80°C freezer at the University of Ottawa, Lees Campus. Samples may be released to co-investigators to analyze with specialized equipment in their labs at CHEO, the University of Calgary, or other labs at the University of Ottawa. Further, samples may be released to external investigators conducting similar investigations as part of secondary analysis, under the guidance of Kristi Adamo, Ph.D. In the event of future research, samples will only be released following the approval of secondary analysis by the Research Ethics Board at the University of Ottawa. Kristi Adamo has been successfully funded for 8 years and will make every effort to ensure the long term sustainability of the biobank. After having completed all of the analyses and stored for the appropriate duration, the University of Ottawa will take all unused blood samples and will destroy them according to their usual method.

How long will I be involved in the study?

Your participation in the study will last approximately 6 months or until delivery. Over this time, you will be required to visit the University of Ottawa-Lees campus, once per trimester of pregnancy, up to a total of 3 times.

What are the potential risks I may experience?

There is little risk to you or your baby by participating in this study.

Blood drawing causes some pain and may cause bruising, bleeding or infections at the site of the needle stick. A nurse or certified phlebotomist has been trained in safely drawing blood. Care will be taken to avoid theses complications.

The walking fitness test will occur in a safe environment and will incorporate the most recent evidence for exercise guidelines during pregnancy. CPR and first aid trained personnel, specially trained to perform exercise testing, will coordinate and monitor the testing. Heart rate will be continually monitored to ensure that you do not reach an unsafe heart rate. Research staff will make sure the treadmill is adjusted properly and will conduct a proper warm-up and cool-down to prevent injuries.

The risk of an adverse event is minimized through the supervision of testing by qualified personnel. In the unlikely event that you experience an injury, medical or psychological crisis (i.e. chest pains, heart attack, panic attack, etc.) during the fitness test, a safety protocol is in place and the university emergency response team will be contacted immediately.

The accelerometer has been approved by Health Canada as a medical device, however, it is small in size and as a result although unlikely, could pose a risk as a choking hazard to children under the age of 3 years old if it were to become detached from its accompanying belt. For this reason, the accelerometer should remain attached to the belt at all times and be worn only by the study participant.

Questionnaires:
You might not like all of the questions that you are asked. You do not have to answer any questions that make you uncomfortable.

If this study uncovers information that might be helpful to your current or future health, the Research Coordinator would offer to discuss these findings with you. The Research Coordinator would first advise you of any risks and benefits of sharing this information with you. If necessary, the investigator will recommend consultation with an appropriate medical professional (e.g. Clinical Psychologist).

Can I expect to benefit from participating in this research study?

The results of these tests may not be directly beneficial to you and your baby but the results will help define the potential role for physical activity in pregnancy, and the knowledge gained from this study may benefit other pregnant women in the future. The results from this study will be shared with health care professionals including general practitioners, obstetricians and gynecologists, exercise and nutrition professionals as well as policy makers and health care planners. At the end of the research study, you are provided the option to receive a summary of your results if you wish. The entire duration of the project is 5 years, therefore it could take over 5 years to receive your results.

Would you like to receive a summary of research results (please check the appropriate box)?

☐ Yes ☐ No

Do I have to participate? What alternatives do I have?

You can choose not to participate in this study. The alternative to this study is not to participate.

Your participation in this study is voluntary. You may decide not to be in this study, or to be in the study now, and then change your mind later without affecting the medical care, education, or other services to which you are entitled or are presently receiving at this institution.

If I agree now, can I change my mind and withdraw later?

You may withdraw from the study at any time without any impact on your current or future care at the Ottawa Hospital, CHEO, Montfort Hospital, or Queensway Carleton Hospital.

- If you withdraw your consent, the study team will no longer collect your personal health information for research purposes and you will no longer be expected to attend the study visits.

Information and samples collected for the study before you cancel this consent may still be used unless you request for them to be destroyed. You have the right to request for them to be destroyed.

What compensation will I receive if I am injured or become ill in this study?

In the event of a study-related injury or illness, you will be provided with appropriate medical treatment and care. Financial compensation for lost wages, disability or discomfort due to an injury or illness is not available. You are not waiving any of your legal rights by agreeing to participate in this study. The Principal Investigator, the Ottawa Hospital, the Montfort Hospital, CHEO, the Queensway Carleton Hospital, the University of Ottawa and the University of Calgary still have their legal and professional responsibilities.

Will I be paid for my participation or will there be any additional costs to me?

You will not be paid to be a participant in the study; however, a parking voucher will be provided to you to cover bus or parking costs for all visits attended with the study team at the University of Ottawa-Lees Campus.

At the end of the study, you will be given the option to receive a general health fitness program developed by a certified exercise physiologist and supervised workout access at our private fitness facility at University of Ottawa – Lees Campus, Behavioural and Metabolic Research Unit. Access will be provided for 3 consecutive months post-partum, starting after you have recovered from the birthing process and within six months of the birth of your baby. Participation in this component is optional and only if you desire.

At the end of the study you will also be given a thank you card with a $50 gift certificate to either a grocery store, book store, or coffee shop (based on your preference) as a token of appreciation for your time to participate in the study visits.

How is my personal information being protected?

- All information and samples collected during your participation in this study will be identified with a unique study number, and will not contain information that identifies you, such as your name, address, etc.
- The link between your unique study number and your name and contact information will be stored securely, password protected and separate from your study records, at The University of Ottawa. The link will not leave the University of Ottawa.
- Any documents or samples leaving the University of Ottawa will contain only your unique study number. This includes publications or presentations resulting from this study.
- Your and/or your baby's medical record will be accessed. However, the information collected, as well as the placenta tissue and blood samples, which leave The Ottawa Hospital, CHEO, Montfort Hospital, or the Queensway Carleton Hospital, will only contain your unique study number.
- Information that identifies you will be released only if it is required by law.
- For audit purposes only, your and your baby's original medical records may be reviewed under the supervision of an investigator and/or their staff by representatives from:
    - the Ottawa Health Science Network Research Ethics Board (OHSN-REB)
    - the Ottawa Hospital Research Institute
    - CHEO Research Ethics Board
    - The Health Sciences and Science REB at the University of Ottawa
    - Montfort Research Ethics Board
    - University of Calgary Conjoint Health Research Ethics Board (CHREB)
    - The Queensway Carleton Hospital Research Ethics Board
  - Research records will be kept for 10 years, as required by the OHSN-REB.
  - At the end of the storage time, all paper records will be shredded and all electronic records will be securely deleted.

A description of this clinical trial will be available at http://www.ClinicalTrials.gov. This website will not contain any information that identifies you. At most, the Web site will provide a summary of results. You can search this Web site at any time.

Do the investigators have any conflicts of interest?

The investigators have no conflicts of interest to declare related to this study.

What are my responsibilities as a study participant?

It is important to remember the following things during this study:

- Ask the research team if you have any questions or concerns.
- Tell the research team if anything about your health has changed.
- You should not eat or drink (except for water) for 8 hours before each visit so that we can obtain fasting blood samples. A snack will be provided immediately following the blood draw.

Who do I contact if I have any further questions?

If you have any questions about this study, please contact Kristi Adamo, Ph.D., at 613-562-5800 x 1009, Dr. Laura Gaudet at 613-737-8899 x 7665, or the study staff at 613-562-5800 x1012 or x7367.

The Ottawa Health Science Network Research Ethics Board (OHSN-REB), CHEO Research Ethics Board (CHEO REB), Hôpital Montfort Research Ethics Board, Queensway Carleton Hospital Research Ethics Board, University of Ottawa Research Ethics Board and the University of Calgary Research Ethics Board have reviewed this protocol. If you have any questions about your rights as a study participant, you may contact the Chairperson of the Ottawa Health Science Network Research Ethics Board at 613-798-5555, extension 16719, the Chairperson of the CHEO Research Ethics Board at 613-737-7600, extension 3272, the Hôpital Montfort Research Ethics Board Manager at 613-746-4621, extension 2221, the Queensway Carleton Hospital Research Ethics Board at (613) 721-2000 extension 1019, or the Office of Research Ethics and Integrity at the University of Ottawa at 613-562-5387.

PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA)

Consent to Participate in Research

- I understand that I am being asked to participate in a research study about physical activity and diet during pregnancy.
- This study was explained to me by _____.
- I have read, or someone has read to me, each page of this Participant **Informed** Consent Form.
- All of my questions have been answered to my satisfaction.
- If I decide later that I would like to withdraw my participation and/or consent from the study, I can do so at any time.
- I voluntarily agree to participate in this study.
- I will be given a copy of this signed Participant **Informed** Consent Form.

Consent for the baby's participation

You accept that your newborn participates in this research conducted by Principal Investigator Kristi Adamo Ph.D.'s research team. I was explained all relevant aspects of the research and my questions were answered to my satisfaction. I was informed that my newborn's participation to this project is voluntary and can cease at any time without any form of penalty. I was given enough time to discuss with my family about the nature and involvement of my newborn in this project. I authorize the archives service to give to the research team only the information from my newborn's medical record mentioned above. I can withdraw my newborn from this project without having to provide a reason.

Please initial:

___ Yes __ No I realize that my participation is voluntary and I am free to withdraw from the study at any time.

___ Yes __ No Having my blood/DNA and placenta tissue samples stored and used for future ethics approved research on health behaviours (i.e. nutrition & physical activity), pregnancy outcomes and weight regulation.

___ Yes __ No Have my child's cord blood/DNA samples banked and used for future, ethics approved research on health behaviours (i.e. nutrition & physical activity), pregnancy outcomes and weight regulation.

___ Yes __ No To be contacted in the future for follow up studies

Contact Information:

Daytime telephone number(s): _____

Evening telephone number(s): _____

Email address: _____

Alternate Email address: _____

_____ _____ _____

Name of Participant (Print) Signature of Participant Date

PhysicaL ACtivity and diEtary implicatioNs Throughout pregnAncy (PLACENTA)

Assistance Declaration

Was the participant assisted during the consent process? ❑ Yes ❑ No

❑ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, and consent was freely given by the participant/substitute decision-maker.

❑ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that the participant/substitute decision-maker has understood the information translated.

_____	_____	_____
Name of Person Assisting (Print)	Signature	Date

Investigator or Delegate Statement

I have carefully explained the study to the study participant. To the best of my knowledge, the participant understands the nature, demands, risks and benefits involved in taking part in this study.

_____	_____	_____
Investigator/Delegate's Printed Name	Investigator/Delegate's Signature	Date

APPENDIX C: INFORMED CONSENT FORM (CONTROL)

uOttawa

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Faculté des sciences
de la santé

École des sciences de
l'activité physique

University of Ottawa
Faculty of Health Sciences

School of Human Kinetics

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Background Information and Consent Form

CHARACTERIZATION OF MECHANICAL EFFICIENCY AND THERMAL STRAIN IN HEALTHY ADULT WOMEN

Investigator:
Miss Kathryn Denize, MSc Candidate, Faculty of Health Sciences, School of Human Kinetics

Co-investigators:
Miss Pegah Akbari, MSc Candidate, Faculty of Health Sciences, School of Human Kinetics

Ms. Alysha Harvey, Clinical Research Associate, Faculty of Health Sciences, School of Human Kinetics

Supervisor:
Kristi Adamo, PhD, Faculty of Health Sciences, School of Human Kinetics

Information to Participants
Participation in this study is voluntary. Please read this Participant Informed Consent Form carefully before you decide if you would like to participate. Please ask the principal investigator and the study team as many questions as you like.

Why am I being given this form?
You have been invited to participate in this study because you are a woman within the ages of 18 – 40 years old, and have shown interest in learning more about this study.

What is the purpose of this study?
The purpose of this study is to characterize and quantify the mechanical efficiency and thermal responses in a group of healthy women (of childbearing age) undergoing a sub maximal exercise test. Mechanical efficiency is the ratio of external work done by the body to the amount of energy used to do that work. This data is also being assessed in a cohort of pregnant women in the REB approved PhysicAL aCtivity and diEtary implicatioNs throughouT pregnAncy (PLACENTA) study (REB# H11-15-29). The data from the current study in the non-pregnant population will be used as a baseline control.

How is this study designed? What is expected of me?
If you consent to participate, you will be asked to visit the Prevention in the Early Years Laboratory at the University of Ottawa – Lees Campus, at a specific date and time which will be arranged between you and the

study team. We need most participants to come in to the lab only once; a small number of women will need to come in twice (3-months apart), with the potential for a third visit (6 months after the initial visit). Please indicate which option you want to participate in on the last page of this document. The estimated length of the initial visit is 1.5 hours. If you choose to come in a second time, the visit will be 1 hour. Visits will take place in the morning, on a weekday or weekend at a time that is convenient for you.

Visit Details

1. Prior to the visit, you will be asked to undergo an 8 hour fast (no food, only water).

2. A trained research personnel will conduct a Dual-energy X-ray absorptiometry (DXA) scan. A DXA scan is used to measure your body composition (bone density, percentage of fat mass and fat-free mass). Your height and weight will also be measured at this time.

3. After the DXA, you be given a small snack, for example a granola bar, a fruit and some juice.

4. With your assistance, we will then outfit you with 4 skin temperature sensors and a heart rate monitor. Once complete, your weight will be taken again on a precise scale.

5. You will rest in a semi-reclined position in a "room-temperature" (thermo-neutral) room for a 20-minute period to get a resting measure. Before this, you will be fitted with a mask to measure the oxygen and carbon dioxide content in your breath, and have your core temperature measured with an ear thermometer.

6. Following rest, you will complete a walking exercise test on a treadmill. You will walk at a pace of 2 mph, and the incline of the treadmill will increase every 3 minutes for approximately 24 minutes. You can choose to stop at any point. We will measure your heart rate and stop the test before you reach 85% of your maximum estimated heart rate.

If you choose to come in for the follow-up visit(s), the same protocol will be repeated.

Possible benefits and risks associated with participating in this study

You will not directly benefit from participating in this study. The results from this study (including the DXA and exercise test) will be provided to you free of charge. This information can be used to understand your current state of health and used to estimate the risk of developing health problems. The results from this study will be used as a control comparator to the pregnant women from the PLACENTA study, since it is not feasible to collect this data prior to conception. The data will provide a means of determining whether mechanical efficiency and thermal strain of pregnant women are elevated above that expected for their given age and body size in a non-pregnant state.

<u>Exercise Test</u>

There are minimal risks associated with participating in this study. The walking fitness test will occur in a safe environment and will incorporate the most recent evidence for exercise guidelines during pregnancy. CPR and first aid trained personnel, specially trained to perform exercise testing, will coordinate and monitor the testing. Research staff will make sure the treadmill is adjusted properly and will conduct a proper warm-up and cool-down to prevent injuries.

<u>DXA</u>

Measures of body fat (DXA) present minimal risk. There is a small level of exposure to X-ray by the DXA machine; it exposes you to minimal radiation, equivalent to 1/20th of the radioactivity received over an 8-hour period in the sunlight.

Alternatives and right to withdraw from the evaluation process:
Participation in this study is completely voluntary. You have the right to refuse any activities or questions asked of you at any time. You can decide to take part in this study now and change your mind later without any negative repercussions. If you withdraw your consent, the study team will no longer collect personal data and use for research purposes.

Confidentiality of Data Collected

- All information and samples collected during your participation in this study will be identified with a unique study number, and will not contain information that identifies you, such as your name, address, etc.
- The link between your unique study number and your name and contact information will be stored securely, password protected and separate from your study records, at The University of Ottawa. The link will not leave the University of Ottawa.
- Any documents or samples leaving the University of Ottawa will contain only your unique study number. This includes publications or presentations resulting from this study.
- Research records will be kept for 5 years, as required by the uOttawa REB.
- At the end of the storage time, all paper records will be shredded and all electronic records will be securely deleted.

Costs and Compensations
You will not be paid to be a participant in the study; however, a parking voucher will be provided to you to cover parking costs onsite, or a bus pass will be provided if preferred, for the visit attended with the study team at the University of Ottawa-Lees Campus.

Conflict of Interest
There are no conflicts of interest to declare related to this study.

Contacts
The Research Ethics Board at the University of Ottawa could have access to study data. Should you have any questions about the study, please contact Kathryn Denize or Pegah Akbari at 613-562-5800 extension 7367, or Dr. Kristi Adamo at 613-562-5800 extension 1009. If no one answers, please leave a message and we will get back to you. The ethical aspects of this study have been reviewed and approved by the University of Ottawa Research Ethics Board. This committee includes a group of professionals who review all human research at the institute. Their goal is to protect the welfare and rights of people involved and they by no means replace the judgement of your choices and decisions that are best for you.

You may contact the Office of Research Ethics and Integrity at 613-562-5387 or via email at ethics@uottawa.ca regarding participants' rights in research studies, but please be aware that this board member cannot provide any health-related knowledge about this study.

Thank you for taking the time to consider this study. We invite you to read, complete and sign the consent form on the following page. There are two copies of the following document, one which you will keep, and the other which we will keep on file.

Participant Consent Form for Participation in the "Characterization of mechanical efficiency and thermal strain in healthy adult women" Study

- I understand that I am being asked to participate in a research study about mechanical efficiency and thermal strain.
- This study was explained to me by _____.
- I have read, or someone has read to me, each page of this Participant Informed Consent Form.
- All of my questions have been answered to my satisfaction.
- If I decide later that I would like to withdraw my participation and/or consent from the study, I can do so at any time.
- I voluntarily agree to participate in this study.
- I will be given a copy of this signed Participant Informed Consent Form.

☐ I agree to participate in one study visit only.

☐ I agree to be contacted for the follow-up visit(s).

_____	_____
Name of Participant	Date of Birth (DD/MM/YY)
_____	_____
Signature of Participant	Date (DD/MM/YY)
(_____)_____	_____
Telephone	Email address

Investigator or Delegate Statement

I have carefully explained the study to the study participant. To the best of my knowledge, the participant understands the nature, demands, risks and benefits involved in taking part in this study.

_____	_____	_____
Investigator/Delegate's Printed Name	Investigator/Delegate's Signature	Date

APPENDIX D: MODIFIED HALO SUBMAXIMAL EXERCISE TEST PROTOCOL

Ottawa Hospital Research Institute / Institut de recherche de l'Hôpital d'Ottawa — uOttawa — Montfort Hôpital universitaire Academic Hospital — CHEO RESEARCH INSTITUTE | INSTITUT DE RECHERCHE

Date (dd/mm/yyyy) / / 20

HALO Submax Protocol

ID number PLA T

DOB (MMM-YY): **Age:**	**Height:** **Weight:** **Pre-instrumentation:** **Instrumented:**	**Predicted HRmax:** **85% HRmax:**
Room Temp: _____ °C	**Relative Humidity: _____ %**	**Air Pressure: _____ psi**

Time (min)	Test Time (sec)	Speed (mph)	Grade %	HR (bpm)	RPE / TS	Core T°
Resting and seated						
0-1						
1-2						
2-3						
3-4						
4-5						
5-6						
6-7						
7-8						
8-9						
9-10						
10-11						
11-12						
12-13						
13-14						
14-15						
15-16						
16-17						
17-18						
18-19						
19-20						

Time (min)	Test Time (sec)	Speed (mph)	Grade %	HR (bpm)	RPE / TS		Core T°
Begin walking							
Warm-up							
0-4	**-4**	2	0				
Commence Test							
4-7	**30 (0:30)**	2	2				
	60 (1:00)	2	2				
	90 (1:30)	2	2				
	120 (2:00)	2	2				
	150 (2:30)	2	2				
	180 (3:00)	2	2				
7-10	**30 (0:30)**	2	4				
	60 (1:00)	2	4				
	90 (1:30)	2	4				
	120 (2:00)	2	4				
	150 (2:30)	2	4				
	180 (3:00)	2	4				
10-13	**30 (0:30)**	2	6				
	60 (1:00)	2	6				
	90 (1:30)	2	6				
	120 (2:00)	2	6				
	150 (2:30)	2	6				
	180 (3:00)	2	6				
13-16	**30 (0:30)**	2	8				
	60 (1:00)	2	8				
	90 (1:30)	2	8				
	120 (2:00)	2	8				
	150 (2:30)	2	8				
	180 (3:00)	2	8				
16-19	**30 (0:30)**	2	10				
	60 (1:00)	2	10				
	90 (1:30)	2	10				
	120 (2:00)	2	10				
	150 (2:30)	2	10				

	180 (3:00)	2	10				
19-22	**30 (0:30)**	2	12				
	60 (1:00)	2	12				
	90 (1:30)	2	12				
	120 (2:00)	2	12				
	150 (2:30)	2	12				
	180 (3:00)	2	12				
22-25	**30 (0:30)**	2	14				
	60 (1:00)	2	14				
	90 (1:30)	2	14				
	120 (2:00)	2	14				
	150 (2:30)	2	14				
	180 (3:00)	2	14				
Rec.	**0-1**	1	0				
Rec.	**1-2**	1	0				

Comments:

Post- Trial Weight: **(Still instrumented)** **_____ Kg**	**Description of Clothing:**

APPENDIX E: INDIRECT CALORIMETRY PROTOCOL FOR DATA COLLECTION AND ANALYSIS

*Please note the Chamber is a shared lab space and therefore should always be left the way it was before use. Ensure inside chamber is clean and desk area is organized. Booking of chamber should be done by booking r-Lees043 in an Outlook calendar invite.

Setting up:

Open ExpeData on the laptop (yellow icon), then:

- In the top select “Acquire” and then “Setup Data Acquisition”
- In pop-up window, press “Re-Scan Comm Ports” and then in the Universal Interface II (UI2) box, select “COM6” from the drop down menu. Press “Use the UI2” Press “OK”
- On the bottom left of 'Acquisition Parameter Box' select 'Channels' in the top menu, and click 'Set-Up', select the Protocol Folder and find file named Placenta_longertime.stp
- Check each channel: O2: A=19, B=0.5 // CO2: A=0, B=0.4 // Flow: A=0, B=100 // WV: A=0, B=1
- Check the large green check mark at the bottom right corner

Use the O2 (20.95%), CO2 (5.0%) gas mix from the gas bottles (against the wall).

Turn on the gas mix from the gas bottle until you feel a light air flow coming from the “Tee-Manifold” tube.

Take the line from the gas bottle and connect the "Tee-Manifold" to the **Chamber** port on the FMS.

Make sure the switch selecting between "Chamber" and "Baseline" is set to "Chamber" (Please make sure it is pushed all the way down, and not clicked in the middle).

Press the Red play button, in the top left corner, on the ExpeData software.

Now that the gases are being measured, complete the following steps while gas is stabilizing.

Calibration:

<u>Main Display on FMS</u>
Confirm 'Sub-Sample Flow Rate' it set to 250 ml/min. (On the FMS, turn “MODE” knob until an arrow appear next to “FLOW SET”. Use the “ADJUST” knob and turn until you reach “250 ml/min”, and press enter.)

Confirm 'Flow Control' is set to 'Closed Loop'. (On the FMS, turn 'Mode' button below Gas Analysis screen until you reach 'FLOW CONTROL IS'. If it is not set to 'CLOSED LOOP', use the 'ADJUST' knob to change it. Confirm by pressing 'ENTER' button.

Confirm the 'O2 and CO2 units are set to “BP compen%”. (On the FMS, turn “MODE” knob until you reach “Gas Units”. If not set to “BP compen%”, turn “ADJUST” knob until “BP compen%” appears and press “ENTER” to confirm.

Confirm O2 and CO2 Signal Averaging is set to 2.5s. (On the FMS, turn “MODE” knob until you reach “AVERAGE O2”. Make sure it reads “02.5s”. If not turn “ADJUST” button until you it ready “02.5s”. Turn “MODE” knob until you reach “AVERAGE CO2”. Make sure it reads “02.5s”. If not turn “ADJUST” button until you it ready “02.5s”.)

Water Vapour Display on FMS

Confirm that 'VWP Signal Averaging: CHOOSE LP FILTER = 1.0 sec'. (On the FMS, under the Water Vapour output, turn “MODE” button below the VWP analyzer screen until you reach CHOOSE LP FILTER. If not set to 1.0 sec, use “ADJUST” knob to change it. Confirm by pressing the “ENTER” button).,

Confirm that 'VWP Analog Output: SET ANALOG OUTPUT 0 – 5 kPa. (On the FMS, under the Water Vapour screen,' turn “MODE” button below the VWP analyzer screen until you reach SET ANALOG OUTPUT. If not set to 0-5 kPa, use “ADJUST” knob to change it. Confirm by pressing the “ENTER” button.)

Flow Kits

Confirm that 'Flow Kit O/P Range': 0-5V = 0-0500 L/MIN (On the Flow Kit, turn “MODE” button until “O/P Range: 0-5V” appear. If not set to 0-0500 L/MIN, turn “ADJUST” knob until the correct setting occur. Press “ENTER” to confirm.

Adjusting CO2

Once the CO2 value has stabilized (at least 1 min), turn “MODE” knob until “ADJUST CO2 SPAN” occur. Use the “ADJUST” knob to make the reading on the display match the CO2 value on the gas you are spanning (display should be **0.5000**; get the gas to hover between 4.995 – 5.010). Press “ENTER” to confirm.

Turn “MODE” knob until “FIXED OXYGEN SPAN 20.95%” appears. Press “ENTER” button to set the span oxygen span.

CALIBRATION COMPLETE

Turn off gas (ensure Black knob is tightly closed, then wait for excess pressure to release, then close the large blue knob (so its “out/loose”) and the small blue knob (so its “in/tight”).

Close recording on ExpeData (do NOT save), and then click “OK” again to open up a new recording.

** Before collecting samples, ensure that the Flow Kits are set to the appropriate flow rate for your data collection (for PLACENTA the top flow kit is set at 100 L/min for rest and 200 L/min for exercise; the bottom flow kit is always at 200 L/min)

Data Collection:

Record the RA outside of the chamber and the 2 RAs inside the chamber

Run an ambient measure first of chamber air by moving the switch to “Baseline” (ensure it moves 2 clicks, 1 click will leave in the middle). Let ambient run for ~5 minutes and then PAUSE. Turn switch back to “Chamber” (ensure it moves 2 clicks, 1 click will leave in middle). Ensure top flow kit is set to 100 L/min.

When participant beings resting measure, hit PLAY. Record flow rate from the LifeWind.

When participant stops resting measure, hit PAUSE. (Now, change the top flow kit to 200 L/min)

When participant beings warm-up, hit PLAY.

When participant changes between stages, place a MARKER.

When participant is finished cool down, place a MARKER. Then, move the switch back to “Baseline” to get a (~5 min) post-test ambient measure of chamber air.

Save the file as “PLA###_DD/MM/YYY”.

Data Analysis:

Using the developed spread sheet to analyse the data in conjunction with the ExpeData software. The darker shaded cells are where you need to input data; the lighter shade cells contain equations.

Collect the most stable 1-min measurement from rest (ensure you collect the same 1-min sample for both CO2 and O2). Collect the last minute of each stage of exercise. You will need to do this for all variables:

- BP
- BP-WV
- WV
- FR (ATPS) – taken from the LifeWind system
- AveO2% and AveCO2% for Inspired (pre-test ambient CO2 and O2)

- AveO2% and AveCO2% for Expired (rest, Stages 1-7).

Check RER value – value should be between 0.84 and 0.90. *Any values below 0.7 and above 1.0 are absolutely incorrect and you must re-assess your analysis. Values between 0.7-0.8 and above 0.95 should be questioned for validity (higher RER can be seen since our participants eat prior to the trial). Usually relates to CO2 values being off, as small adjustments in these values cause large changes in outcomes.*

APPENDIX F: CALIBRATION OF SYSTEMS

PROPANE VALIDATION

To ensure the accuracy of our $\dot{V}O_2$ and $\dot{V}CO_2$ measurements, we conducted a propane burn validation test. Propane was burned for 30-minutes, with mass measured pre- and post- of the test. The mass was used to calculate expected $\dot{V}O_2$ and $\dot{V}CO_2$ based on their respective stoichiometry's, which was then compared to the values obtained during the combustion test. The differences between expected and measured values for $\dot{V}O_2$, $\dot{V}CO_2$ and RER were <10%.

TREADMILL CALIBRATION

The speed and incline of the treadmill were calibrated to ensure accurate measurement of completed W_{ext}.

To calibrate the speed, the following steps were conducted:

1. Measured the length of the belt, by marking the belt with a piece of tape and marking each consecutive metre with tape until reaching the starting point.
2. Counted the number of revolutions completed over 60-seconds while the treadmill was at a speed of 3.2 km/h
3. Calculated total horizontal distance travelled by multiplying measured belt length by revolutions per minute

$$distance = speed\ x\ time$$

where distance is in metres, speed is of the treadmill in m/s, and time is in seconds for one stage of the protocol.

To calibrate the incline of the treadmill, we completed the following:

1. Measured the height of the treadmill from the initial position (0% grade), and then at every 2% grade increment until 14%.
2. Subtracted the initial position from the measured distance to get delta height.
3. Took the tangent of that height to get the true % incline.