MATERNAL CARDIOMETABOLIC STATUS IN EARLY PREGNANCY

MATERNAL CHARACTERISTICS ASSOCIATED WITH CARDIOMETABOLIC STATUS IN EARLY PREGNANCY

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ABSTRACT

Rationale & Background: During pregnancy, cardiometabolic adaptations occur to sustain fetal growth. Disruptions in maternal cardiometabolic status may arise related to maternal adiposity, dietary deficiencies or excesses, or sedentary behaviours in pregnancy. Clinically, maternal cardiometabolic dysfunction is associated with adverse health outcomes in both mothers and their offspring. We aimed to determine: 1) the contribution of maternal adiposity, diet and physical activity to maternal cardiometabolic status in early pregnancy using biomarkers of lipid and glucose profiles; 2) whether maternal adiposity measured by 4-site sum of skinfold thickness (SFT) or bioelectrical impedance analysis (BIA) yielded similar strength of association with cardiometabolic status.

Study Design: Maternal blood samples, anthropometric and body adiposity, dietary and physical activity measures were collected from a subset of pregnant women in early pregnancy (12-17 wk gestation) prior to randomization to the Be Healthy in Pregnancy RCT. Blood samples were analyzed for fasting glucose, insulin, triglycerides, leptin, adiponectin, and C-reactive protein (CRP). Maternal adiposity was assessed by pre-pregnancy body mass index (pBMI) and two indirect quantitative measures of % body fat (BIA and 4-site SFT).

Results: Of the 91 subjects (mean age= 31 ± 4 y), 46.2% were overweight/obese by pBMI. For both SFT and BIA, % body fat was positively associated with fasting glucose, insulin, triglyceride, leptin, and CRP concentrations, and negatively associated with adiponectin concentration, although the strength of the associations was greater for SFT

than BIA. After adjustment for confounders, maternal adiposity remained significantly associated with all cardiometabolic biomarkers, except for adiponectin and CRP. Dietary polyunsaturated: saturated fat ratio, energy expenditure, high activity level, age, ethnicity and parity were significantly associated with some of the biomarkers.

Conclusion: Maternal adiposity was predominantly associated with leptin, insulin, and glucose status in early pregnancy although dietary fat, energy, activity level, age, ethnicity and parity were also significantly associated with some biomarkers. Body fat estimated by SFT or BIA are generally comparable for use as a screening tool for cardiometabolic dysfunction in early pregnancy. In the clinical setting, BIA may be more easily adopted as it is faster and requires fewer technical skills by the operator than SFT measures.

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LIST OF ABBREVIATIONS

ADP – air displacement plethysmography

APrON – Alberta Pregnancy Outcomes and Nutrition

ATP – adenosine triphosphate

BHIP – Be Healthy in Pregnancy

BIA – body impedance analysis

BMI – body mass index

CRP – C-reactive protein

CV – coefficient of variation

DASH – Dietary Approaches to Stop Hypertension

DOHaD – Developmental Origins of Health and Disease

DXA – dual-energy X-ray absorptiometry

EDTA – ethylenediaminetetraacetic acid

ELISA – enzyme-linked immunosorbent assay

FAMILY – Family Atherosclerosis Monitoring In earLY Life

FFQ – food frequency questionnaire

FFM – fat-free mass

FM – fat mass

G-6-P – glucose-6-phosphate

GDM – gestational diabetes mellitus

GK – glycerol kinase

GWG – gestational weight gain

H_2O_2 – Hydrogen peroxide

HOMA-IR – Homeostatic Model Assessment of Insulin Resistance

HRLMP – Health Sciences Regional Laboratory Medicine Program

IL-6 – interleukin 6

IOM – Institute of Medicine

LCA – latent class analysis

LGA – large for gestational age

LED – light emitting diode

MVPA – moderate-to-vigorous physical activity

METs – metabolic equivalent of activity

MRI – magnetic resonance imaging

NAD – nicotinamide adenine dinucleotide

NADH – nicotinamide adenine dinucleotide reduced

PA – physical activity

pBMI – pre-pregnancy body mass index

PPARγ – peroxisome proliferator activated receptor γ

QUICKI – qualitative insulin sensitivity check index

RCT – randomized controlled trial

SFT – sum of skinfold thickness

SREBP-1 – sterol regulatory element binding protein-1

Streptavidin-PE – streptavidin-phycoerythrin

TBW – total body water

VLDL – very low-density lipoprotein

CHAPTER 1

INTRODUCTION

CHAPTER 1 – INTRODUCTION

1.1 Clinical problem: Pregravid obesity and excess gestational weight gain

1.1.1 Prevalence in Canadian women

Pregravid obesity and excess gestational weight gain (GWG) are prevalent in Canada and present major clinical challenges in pregnant women. At the national level, a cross-sectional study on pregnancy experiences in Canada determined that over one third of women entered pregnancy overweight or obese and nearly 60% experienced GWG greater than that recommended by the Institute of Medicine (IOM) recommendations for GWG (outlined in Table 1).[1] The Alberta Pregnancy Outcomes and Nutrition (APrON) prospective cohort study reported similar statistics with a greater number of overweight and obese women (80%) gaining excess weight during pregnancy.[2] Likewise in the Hamilton region data from the Family Atherosclerosis Monitoring In earLY life (FAMILY) birth cohort demonstrated that over 50% of women were entering pregnancy with a pre-pregnancy body mass index (pBMI) of >25.0 kg/m^2, and over 50% exceeded the IOM guidelines for GWG.[3]

Maternal adiposity is influenced by multiple factors including genetics, sociocultural factors, and an environmental milieu that is in turn affected by diet quality, physical activity, environmental toxins, and energy expenditure versus intake. Both dietary deficiency and excess prior to conception and during pregnancy play a significant role in fetal programming which in turn contributes to future disease risk in offspring.[4] Dietary nutrient inadequacy in pregnancy is evident from a meta-analysis that demonstrated women living in developed countries are not meeting

recommendations for energy intake and certain macronutrients during pregnancy.[5] These findings were confirmed in a cohort of pregnant women living in Québec, Canada.[6] In regard to energy intake in pregnancy, inadequacies present clinical challenges as total energy intake has been positively associated with GWG[7]; both overconsumption and large portion sizes of energy-dense foods in pregnancy are thought to be contributing factors.[8]

Physical activity (PA) level during gestation also plays an important role in maternal and fetal health as it aids women in meeting GWG goals.[9] However, many women become less active during pregnancy compared to pre-pregnancy due to normal physiological changes which limit their ability to participant in PA (e.g. skeletomuscular and cardiopulmonary)[10] in consort with social and psychological factors.[11] The imbalance between energy intake and energy expenditure during pregnancy is a contributing factor for poor adherence to GWG recommendations.[9]

Table 1: Reference values for adequate GWG according to IOM recommendations, based on pBMI[12,13]

Pre-pregnancy BMI (kg/m^2)		**Recommended gestational weight gain**
Underweight	<18.5	12.5-18 kg (28-40 lbs)
Normal weight	18.5-24.9	11.5-16 kg (25-35 lbs)
Overweight	25.0-29.9	7-11.5 kg (15-25 lbs)
Obese (all classes)	≥30.0	5-9 kg (11-20 lbs)

1.1.2 Adverse health outcomes in mothers and their offspring

The high prevalence of pregravid obesity and excess GWG presents clinical challenges as such conditions are associated with serious adverse health outcomes in both mothers and their offspring. Maternal adiposity is associated with changes in maternal carbohydrate and lipid metabolism, body composition, and immunity above and beyond the normal physiological adaptations that occur in pregnancy. Consequently, obese women have a greater risk of gestational diabetes mellitus (GDM) and preeclampsia.[14] Such conditions are associated with adverse obstetrical outcomes such as spontaneous miscarriage, adverse birth outcomes (e.g. preterm birth, stillbirth), congenital anomalies (e.g. spina bifida, neural tube defects), and higher rates of Caesarean section.[14] Additionally, there are long-term consequences for women with pregravid obesity and metabolic dysfunction in pregnancy; for instance, increased risk of metabolic syndrome (e.g. type II diabetes)[15] and coronary heart disease.[16]

Pregravid obesity and excess GWG are the strongest maternal characteristics associated with offspring obesity[17,18] and childhood metabolic dysfunction.[19] Maternal metabolic dysfunction in pregnancy is associated with offspring obesity and cardiometabolic risk factors later in life.[20] Offspring with greater neonatal adiposity have increased risk of cardiovascular disease, type II diabetes, and neurodevelopmental disorders.[21] Such evidence is concordant with the Developmental Origins of Health and Disease (DOHaD) hypothesis that the early life environment plays a key role in predicting disease risk in offspring.[22]

Adverse metabolic adaptations associated with maternal obesity can disrupt normal placental physiology resulting in heavier placentas and fetal overgrowth. Obese compared to normal weight women have significantly heavier placentas at birth,[23] which is strongly correlated with greater birth weight and higher fat mass (FM) in neonates.[24] Early placental growth and gene expression, as well as placental function in late pregnancy, are affected by the in utero metabolic environment established by the mother.[25] Both macrosomia (birth weight ≥4000g)[26] and large for gestational age (LGA) (birth weight >90% percentile for gestational age)[27] are associated with maternal insulin resistance and hyperinsulinemia during pregnancy.[14]

Maternal overweight and obesity result in the development of adverse inflammatory profiles which can stimulate increased placental glucose and amino acid uptake via upregulation of placental transporters.[28] These alterations to maternal-fetal nutrient pathways are associated with risk of fetal overgrowth leading to macrosomia and adiposity in the infant.

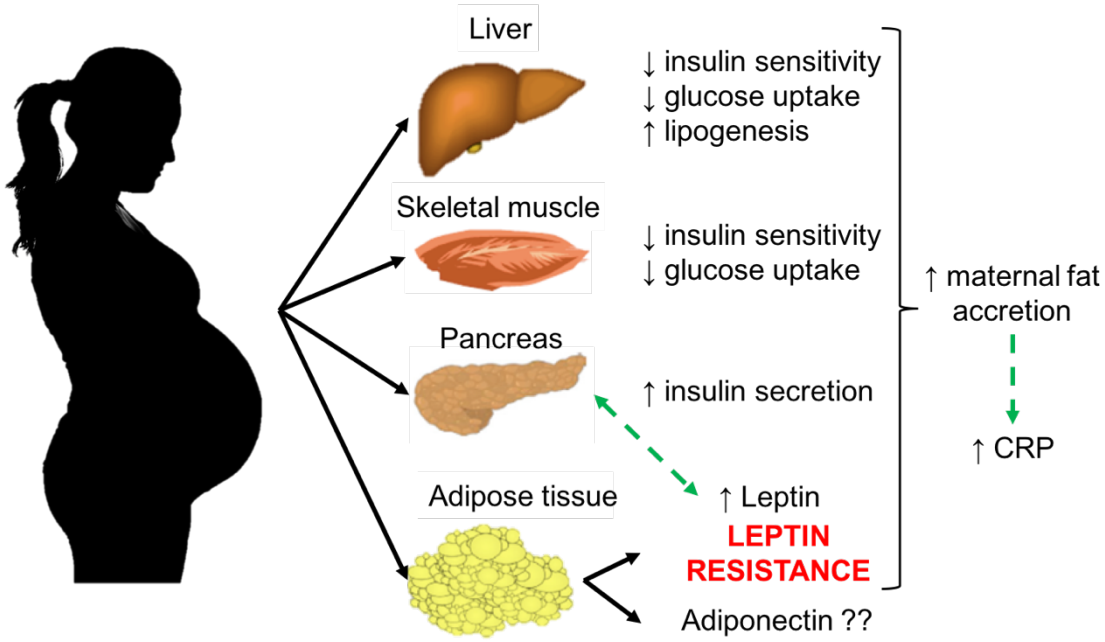
1.2 Normal cardiometabolic adaptations in early pregnancy

1.2.1 Glucose homeostasis and insulin sensitivity

During pregnancy, several metabolic adaptations occur naturally in response to the needs of the feto-placental unit. The first two trimesters can be viewed as an anabolic state that promotes maternal fat accretion (Figure 1), a critical source of energy to sustain maternal and feto-placental demands in late pregnancy. Fasting glucose and hepatic glucose production remain unchanged in early gestation.[29] However, alterations to insulin sensitivity in early pregnancy are thought to

contribute to increased growth of maternal fat depots. Although fasting insulin concentrations remain constant, insulin secretion is increased which is most likely attributed to compensation for insulin sensitivity decline in early pregnancy.[29] The decline in hepatic and peripheral insulin sensitivity results in decreased glucose uptake by the maternal liver and skeletal muscle. This metabolic milieu in addition to the influence of cortisol, estrogens, and progestins stimulates lipogenesis and maternal fat accretion.[30] Specifically, insulin is known to stimulate lipogenesis via the transcription factors sterol regulatory element binding protein-1 (SREBP-1) and peroxisome proliferator activated receptor γ (PPARγ), and inhibit whole body lipolysis.[31,32]

Figure 1: Maternal adaptations in metabolism and body composition in early pregnancy



**Adopted and modified from Park and Ahima Metabolism. 2015;6(1):24-34*

1.2.2 Lipid profile

In addition to adaptations in glucose homeostasis and insulin sensitivity, lipid metabolism changes in early pregnancy to promote maternal fat accretion. For example, de novo lipogenesis and lipoprotein lipase activity are augmented during the first two trimesters leading to increased deposition of lipids in maternal adipose tissue.[33] Lipoprotein lipase activity increases hydrolysis of circulating triglyceride-rich chylomicrons and very low-density lipoproteins (VLDLs) in human pregnancy, which releases non-esterified free fatty acids and glycerol for uptake by adipose tissue.[34] In contrast, fasting triglyceride concentrations remain relatively unchanged in early pregnancy from pregravid values.[35]

1.2.3 Adipokines – leptin and adiponectin

Proteins secreted by adipose tissue known as adipokines may influence metabolic adaptations during pregnancy. Leptin, the satiety hormone, which is produced in adipocytes as well as the placenta during gestation, is elevated in early pregnancy compared to pregravid values.[36] This adipokine is mainly associated with fetal development and proper functioning of the placenta[37] whereas its role in metabolic adaptation in early pregnancy remains to be elucidated. Interestingly, hyperphagia or excessive hunger often observed in pregnant women contradicts the sating effects of leptin.[38] This suggests that the development of central leptin resistance commences in early pregnancy[37] thus diminishing the suppression of lipogenesis and stimulating increased energy intake.[39]

Leptin may promote the rise in insulin secretion observed in pregnancy as both Ob-Rb and Ob-Ra (long and short leptin receptor isoforms) are expressed in pancreatic β-cells.[39] However, whether leptin inhibits or increases insulin secretion and vice versa remains controversial thus the directionality of this relationship requires further elucidation. The combination of central leptin resistance and increased insulin secretion leads to increased nutrient availability and lipogenesis stimulation which promotes maternal fat accretion in early pregnancy.

Adiponectin, an adipokine produced exclusively in white adipose tissue, may play a role in the metabolic adaptations characteristic of pregnancy. Maternal fat accretion in early pregnancy is thought to reduce circulating adiponectin concentrations as gestation advances which in turn decreases insulin sensitivity and facilitates lipolysis to support nutrient demands of the developing fetus in late pregnancy.[40] In normal pregnancy, reduced adiponectin release is associated with fat accumulation in adipocytes.[41] However, changes in concentration of adiponectin across pregnancy are inconsistent with some studies reporting adiponectin decline as pregnancy progresses[41-43], while others found higher concentrations in pregnancy compared to postpartum.[44-46] In non-pregnant adults with overweight and obesity, hypoadiponectinaemia is associated with decreased insulin sensitivity and increased lipolytic activity.[47] Such metabolic adaptations are also observed in normal pregnancy; thus, the development of hypoadiponectinaemia with progressing gestation is biologically plausible. However, evidence for these associations in pregnant women is contradictory as a study by Catalano *et al.* found that adiponectin

decline in the third trimester was more related to alterations in insulin sensitivity than lipid metabolism whereas Ritterath *et al.* concluded the opposite; adiponectin decline was correlated with lipid metabolism rather than carbohydrate metabolism.[42,48]

1.2.4 Inflammatory profile – C-reactive protein

Beyond the profound metabolic adaptations observed in healthy pregnancy, the maternal inflammatory profile is significantly altered. The first trimester is characterized as pro-inflammatory and transitions to an anti-inflammatory state as gestation advances.[49] As pregnancy is akin to an obesity-like state and obesity is known to promote chronic low-grade inflammation, this knowledge supports the alterations to the inflammatory profile occurring during pregnancy.

C-reactive protein (CRP), an acute phase protein mainly produced in the liver when stimulated by inflammatory mediators such as interleukin 6 (IL-6), is a marker of low-grade systemic inflammation.[50] In pregnancy, circulating concentrations of CRP are elevated compared to non-pregnant values[50-52] but the pattern of change across gestation is not well resolved.[50-56] Further studies are needed to elucidate whether CRP concentration is steadily elevated across pregnancy or progressively increased. Maternal fat accretion is thought to contribute to elevated CRP in pregnancy (Figure 1) as adipocyte volume has been positively associated with CRP levels in non-pregnant subjects.[57] The progressive increase in estrogen concentration during pregnancy may increase serum protein production in the liver which may also play a role in elevated CRP during pregnancy.[51,52]

1.3 Maternal characteristics influencing cardiometabolic status in pregnancy

1.3.1 Adiposity

1.3.1.1 Exaggerated glucose, insulin, and triglyceride profiles

Excess adiposity is associated with adaptations in maternal metabolism and cardiometabolic biomarker profiles that extend beyond the normal changes associated with pregnancy in normal weight women. With respect to glucose homeostasis and insulin sensitivity, pregravid obesity is associated with less of a decline in fasting glucose in pregnancy, and in severely obese women no measurable decline.[58] In contrast, adaptations in lipid metabolism are uniform in all pregnant women regardless of adiposity and glucose tolerance; pregravid lipogenesis shifts to lipolysis in late pregnancy.[59] Hyperlipidemia and increased VLDL triglyceride concentrations observed in normal pregnancy are exaggerated in obese pregnant women.[34] Taken together, it appears that the pregravid metabolic milieu has a greater influence on lipid profile changes in pregnancy than metabolic adaptations such that the higher post-prandial circulating concentrations of glucose, lipids, and amino acids normally observed in pregnancy are exaggerated in obese women.[59]

1.3.1.2 Abnormal adaptations in leptin and adiponectin status

Adipokines are influenced by maternal adiposity during pregnancy. Leptin is elevated in women with greater maternal adiposity.[60] As a preliminary project to this thesis, data from the FAMILY study were analyzed to assess the association between maternal leptin and adiposity in late pregnancy. We observed that maternal serum leptin in the third trimester of pregnancy was higher in women with a pBMI of

overweight and obese compared to normal weight pBMI and that it was positively correlated with sum of skinfold thickness (SFT) of triceps and subscapular sites (See Appendix 1). This supports previous observations of leptin status and maternal adiposity in pregnancy.[60-63]

Maternal adiposity in pregnancy is also suggested to impact adiponectin. Circulating adiponectin and pBMI are inversely correlated.[64-66] Whether maternal FM is a predictor of adiponectin concentration during pregnancy is uncertain. An inverse correlation between adiponectin and maternal FM in pregnant women was observed in three studies.[64,67,68] In contrast, a more recent study found that neither total nor high-molecular weight adiponectin concentrations in pregnancy were associated with total body FM or abdominal FM. Dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI), which are gold-standard methods, were used to measure adiposity rather than the less accurate methods used by previous studies.[69] Future research might clarify these inconsistencies by assessing the association between adiponectin and maternal adiposity measured by multiple methods including DXA or MRI.

1.3.1.3 Adverse Inflammatory cytokine profiles

In addition to the regulation of metabolic processes, adipokines such as leptin and adiponectin can also function as mediators of inflammation. Leptin acts as a pro-inflammatory mediator playing an important role in the regulation of both innate and adaptive immunological processes,[70] whereas adiponectin has anti-inflammatory properties.[71] Although adaptations to the inflammatory profile are expected in normal

pregnancy, abnormal and exaggerated changes associated with excess maternal adiposity may have adverse effects on maternal and offspring health because of the disruption in the delicate balance of inflammatory mediators required to support a healthy pregnancy.[50] In obese non-pregnant individuals, higher circulating leptin and lowered adiponectin may act synergistically to give rise to chronic systemic inflammation. Further elucidation is needed to determine whether these findings are also present in obese pregnant women.

Excess maternal adiposity is associated with abnormally elevated circulating CRP in pregnancy.[50,53] A possible synergistic relationship between the normal elevations in CRP observed during pregnancy and obesity is thought to contribute to such adaptations in the inflammatory processes.[50] However, the exact mechanisms are unknown. Higher circulating CRP in overweight and obese women may be attributed to exaggerated insulin resistance[70] and circulating IL-6 concentration[50] associated with greater maternal FM. Moreover, it is estimated that 80% of women who enter pregnancy with greater FM (i.e. pBMI>25.0 kg/m^2) are exceeding IOM recommendations for GWG.[2] Together, pregravid obesity and excess GWG could cause greater elevations in circulating CRP in overweight and obese women compared to those with normal pBMI.

1.3.2 Dietary patterns

Healthy dietary patterns in pregnancy may contribute to favourable cardiometabolic status, however, studies evaluating this relationship are limited. Martin *et al.* recently investigated the association of maternal dietary patterns with

cardiometabolic markers at 26-29 weeks gestation using data from the Pregnancy, Infection, and Nutrition prospective cohort study in the U.S.[72] Dietary patterns were assessed using latent class analysis (LCA) and the Dietary Approaches to Stop Hypertension (DASH) diet which consists of higher intakes of fruits, vegetables, nuts and legumes, low-fat dairy, and whole grains rather than red and processed meats, sugar-sweetened beverages, and food items with high sodium. Maternal cardiometabolic markers included glucose, insulin, insulin resistance (Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)), triglycerides, and cholesterol.

The DASH scores were negatively associated with maternal insulin, HOMA-IR, and triglycerides. By means of LCA, it was determined that consumption of fruits, vegetables, whole grains, low-fat dairy, breakfast bars, and water was associated with better maternal insulin and HOMA-IR status. Fasting glucose was negatively associated with both LCA and DASH diet scores but these associations were no longer significant following adjustment for pBMI. Overall, the benefits of healthy dietary patterns on glucose and insulin sensitivity are supported by previous studies in pregnant women.[73,74] Notably, one of these studies only included women diagnosed with GDM in their study group and did not consider the effects in healthy pregnant women.[73] No further studies have assessed the benefits of healthier dietary patterns on triglyceride concentrations in a pregnant population.

Research on the influence of dietary patterns on adipokine status in pregnancy is limited to a single study. Total fat intake was positively correlated with leptin concentration in the first trimester whereas an inverse correlation with adiponectin

was observed.[75] A limitation of these findings is the relatively small sample size (n=49). Regardless, this potential relationship between dietary fat and adipokine status during gestation presents clinical concerns because pregnant women living in developed countries including Canada are exceeding recommendations for total fat intake during pregnancy.[5,6] Thus, further research is warranted to clarify whether a true relationship between dietary fat and adipokines exists.

Multiple studies have assessed the influence of maternal diet on CRP concentration in pregnancy but inconsistency exists as to whether a healthier dietary pattern benefits maternal CRP status. A negative association was observed between dietary glycemic load and CRP throughout pregnancy in a prospective cohort study of pregnant women (n=115) receiving prenatal care in Rio de Janeiro, Brazil.[55] CRP was also positively associated with greater protein and cholesterol intake during mid-pregnancy in lean women (after stratifying for body mass index (BMI)) in the Camden Study, a prospective cohort of pregnant women in the US.[76] In contrast, no difference in CRP concentration between control and DASH diet groups was observed in a randomized controlled trial (RCT) investigating the effects of the DASH diet in women with GDM.[77]

1.3.3 Physical activity

The beneficial effects of PA on cardiometabolic health are well-established in non-pregnant populations. A meta-analysis of RCTs in adults found that exercise training significantly improved triglycerides, leptin, insulin and insulin sensitivity, whereas PA was not associated with glucose, adiponectin, and CRP concentrations.[78]

Potential confounding variables including age, sex, and health status were considered. Although the beneficial effects of PA for achieving adequate GWG are known, its impact on cardiometabolic profiles during pregnancy remains unclear.

In pregnant populations, the effects of PA on glucose and insulin sensitivity are summarized in a meta-analysis which found that PA had a slight protective effect against the development of GDM.[79] The RCTs included in the analysis reported inconsistent results which were attributed to low intervention compliance[79] and the relatively high number of health-conscious women (~50%) in the study groups.[80] More recently, the New Life (style) RCT showed similar limitations. [81] Consequently, no significant difference in moderate-to-vigorous physical activity (MVPA) was observed between exercise intervention and control groups; thus, a secondary analysis of data was conducted. MVPA above the median was associated with lower insulin and insulin sensitivity compared to women with MVPA below the median before 15 weeks of pregnancy. PA was not significantly related to metabolic outcomes in mid- to late pregnancy. Conversely, the latest RCT to explore the influence of PA in pregnancy on maternal glucose and insulin sensitivity reported a high level of adherence to the structured and supervised exercise intervention. Cycling exercise commenced in early pregnancy was found to significantly improve insulin sensitivity in mid-pregnancy and reduce the risk of GDM in overweight/obese women.[82]

A beneficial impact of PA on blood lipid profiles has been consistently reported. The OMEGA prospective study found an inverse association between

triglyceride concentration in early pregnancy and PA, a relationship that remained significant following adjustment for age, ethnicity, smoking, parity, BMI, and dietary variables.[83] Subsequent observational studies have reported similar findings.[84,85] The exception is an observational analysis (n=206) among U.S. pregnant women using data from the 2003-2006 National Health and Examination Survey found no effect of PA on triglyceride concentration.[86] One randomized trial has examined cardiometabolic outcomes in response to PA during pregnancy in women with GDM (n=200).[87] Triglycerides were significantly lower in the exercise group compared to the control following adjustment for age, pBMI, metabolic equivalent of activity (METs), and baseline values of triglycerides. Intervention compliance was good with >60% of the exercise intervention groups adhering to PA recommendations.

The evidence for an influence of PA level during gestation on maternal leptin is inconsistent with studies reporting inverse[88,89], positive[90], and null[80,81,91,92] associations. Pregnant women in the two observational studies that found an inverse association were more physically active before and during pregnancy than the average pregnant woman.[88,89] Conversely, a secondary analysis of RCT data found no significant association between MVPA and leptin possibly due to the fact that the sample consisted of pregnant women with low levels of MVPA.[81] Low intervention adherence is a major issue for RCTs with PA interventions in pregnant women and may explain the null and inconsistent findings. Three RCTs with low intervention compliance found no effect of PA on leptin concentrations in pregnant women.[81,91,92] In contrast, an earlier RCT conducted in New Zealand observed good overall

compliance to the intervention and found a positive association between exercise training initiated in mid-pregnancy and leptin concentration in late gestation.[90]

Evidence for the association between PA and adiponectin during pregnancy is consistent but limited to only two studies. Physical activity of moderate to vigorous intensity did not influence adiponectin concentrations in obese pregnant women in a longitudinal study in Amsterdam.[93] This is supported by an earlier RCT in U.S. pregnant women that found no significant difference in maternal adiponectin concentration between the intervention or control groups despite higher PA by self-report in the intervention group.[80]

Physical activity during pregnancy may reduce adverse proinflammatory cytokine profiles associated with preeclampsia and GDM.[92] Observational data consistently suggests an inverse relationship between PA and CRP concentration in pregnant women,[94-96] whereas findings from RCT studies are inconsistent.[87,92,97] The contraindications between studies may arise from the method used to measure PA (i.e. self-reported vs. objectively measured data), small sample sizes, and the assessment of associations occurring at different time points in pregnancy.

1.4 Challenges in measuring maternal adiposity in pregnancy

One challenge with measuring adiposity in pregnancy is that there is no gold-standard tool that is both safe for the fetus and not influenced by the physiological changes in body composition during pregnancy. The commonly used methods that are safe for use in pregnancy include anthropometric (i.e. weight and height, BMI, waist and hip circumference, skinfold thickness) and body composition (i.e.

densitometry, hydrometry, bioelectric impedance analysis (BIA), imaging) measures. In the context of pregnancy, each method is subject to strengths and weaknesses.

Anthropometric measures, predominantly BMI, are used most often as such methods are easy, inexpensive, and efficient for larger sample sizes. Limitations include inter-observer variations and the use of self-reported weight and height both before and during pregnancy.[98] Height measurements can also be affected by normal postural changes as pregnancy progresses and weight measurements cannot distinguish between maternal, fetal and placental contributions. Waist and hip circumference cannot be used after pre-pregnancy and early pregnancy due to increasing abdominal size.

One major issue with anthropometric measures is that they do not directly measure FM. The use of BMI can lead to under- and overestimation of body fat as it is a surrogate measure and cannot distinguish body composition (i.e. fat vs. lean mass).[99] Nor does it consider factors such as age, ethnicity, bone structure and fat distribution which can lead to further misrepresentation of body fat between individuals. For example, one study found a 15% difference in % body fat in two pregnant women with class 1 obesity in addition to women being categorized as overweight and obese with similar % body fat, thus indicating that BMI does not necessarily correspond with body fat.[100] Misclassification may have serious implications for the identification of individuals with increased risk of perinatal complications due to adiposity-related physiological and metabolic dysfunctions.

Skinfold thickness is one approach to quantitatively measure FM, albeit indirect, that is efficient and effective but it also has disadvantages. These include high inter-observer variations, low reproducibility, and unreliability for measuring intra-abdominal fat or central obesity.[98,100] Some studies have also reported that skinfolds have a high susceptibility to overestimate FM in pregnant women due to increased water retention, especially in later gestation.[100] Unlike other anthropometric methods, skinfold thickness does distinguish between the maternal and fetal contributions.

Fat mass can be quantitatively measured using body composition methods such as BIA, densitometry, hydrometry, DXA, and MRI. However, these methods also have shortcomings and most cannot disentangle the maternal-fetal unit. Although BIA is an efficient method for larger-scale studies and easy to perform, values are indirect measures dependent on the algorithm used by each model of scale[101] and are sensitive to shifts in total body water (TBW) changes especially in late pregnancy.[100] Densitometry (i.e. air displacement plethysmography (ADP), hydrostatic/underwater weighing) and hydrometry (deuterium oxide dilution) are more accurate and precise as both directly measure maternal FM in pregnancy, yet both methods are time consuming and not suitable for large-scale studies.[98] Hydrometric measures also require overnight fasting and additional time spent with no food or water intake and minimal activity over the salivary collection period, conditions not ideal for subjects in late gestation.[100]

DXA and MRI are the most accurate direct measures of FM and are considered "gold-standard" methods for measuring body composition in a non-pregnant population. However, DXA is contraindicated in pregnancy because fetal exposure to X-rays is unsafe, and therefore, cannot be used to measure maternal adiposity during gestation.[98,100] Although MRI is safe to perform on pregnant subjects, imaging is expensive and impractical in large-scale studies.[98]

In a previous study that compared various methods for measuring maternal FM in late pregnancy, the authors concluded that ADP, whole body densitometry that measures body composition, was the preferred method.[100] In the study, ADP was well tolerated by subjects and more reliable than hydrometry. Nonetheless, the authors found the use of SFT was optimal for larger field studies or those with limited resources. Measurements of FM estimated from SFT were reasonably close to those measured using DXA, even though increases in TBW may have contributed to overestimation bias in late pregnancy. Percent body fat obtained with BIA had a wider range of values than those estimated with SFT; thus, SFT was deemed more reliable than BIA for determining FM in late pregnancy. Future studies should address this variation in BIA measurements as research protocols are increasingly using emerging techniques such as BIA.

1.5 Study rationale

Few studies have objectively compared multiple methods of body composition measurement in pregnant populations. Most often BMI is used as a surrogate for body fat assessment despite knowledge that it does not truly reflect adiposity.[99] However,

agreement does not exist on the optimal method to quantitatively assess body fat in pregnancy.[100] This study employed two recommended methods to quantitatively measure body fat – SFT and BIA. Our goal was to determine which tool might be the most appropriate method to use as a screening tool for cardiometabolic risk in early pregnancy.

Gaps in knowledge and areas of controversy exist in research concerning the association between maternal characteristics and cardiometabolic biomarker profiles in early pregnancy. First, adiponectin concentration has inconsistently been linked with maternal adiposity depending on whether direct or indirect quantitative methods were employed to measure FM in pregnant women. We assessed the association between maternal adiponectin and two indirect quantitative measures of body fat – SFT and BIA – in early pregnancy to provide clarification on these inconsistencies. Second, the impact of dietary patterns on lipid, adipokine, and inflammatory profiles during pregnancy remains unclear especially regarding the potential relationship between dietary fat intake and adipokines. Furthermore, only a single study has assessed the effect of different types of fat on adipokine status in pregnancy. Because of the detailed dietary analysis from food records of habitual diet in early pregnancy, our study evaluated the effect of energy intake and polyunsaturated: saturated fat intake ratio on leptin and adiponectin. Lastly, evidence is contradictory regarding the beneficial effects of PA level during pregnancy on maternal leptin and CRP status. We examined this relationship to contribute new knowledge since we employed objective methods for measuring habitual PA level in the pregnant women prior to randomization to treatment intervention.

1.6 Objectives and hypotheses

The overall objective was to determine the contribution of maternal adiposity, diet and physical activity to maternal cardiometabolic status in early pregnancy using biomarkers of lipid and glucose profiles. Our hypothesis was that:

1) Greater maternal fat mass will be significantly associated with higher glucose, insulin, triglycerides, leptin, and CRP whereas adiponectin will be decreased. These associations will be attenuated following adjustment for dietary and physical activity factors.

Using baseline maternal cardiometabolic biomarker, adiposity, diet and physical activity data from women enrolled in an RCT, this thesis aimed to determine:

1) whether SFT and BIA were similarly associated with cardiometabolic status in pregnancy
2) the association between adiponectin concentration and maternal adiposity
3) the impact of dietary patterns on lipid, adipokine, and inflammatory profiles during pregnancy. Specifically, we aimed to determine whether a true relationship exists between dietary fat intake and adipokine biomarkers, and the effect of polyunsaturated versus saturated fat intake on adipokine status.
4) the relationship of PA level in pregnancy with maternal adipokine and inflammatory biomarker status.

We hypothesized that:

1) SFT will be more strongly associated with cardiometabolic status than BIA
2) % body fat estimated from both SFT and BIA will be significantly associated with adiponectin
3) healthier dietary patterns will benefit all cardiometabolic profiles except for glucose
4) increased PA will benefit leptin and CRP status, but not adiponectin

As a secondary objective, we evaluated the congruence of two methods to define adiposity and the extent to which BMI misrepresents classification of adiposity status by quantitative body fat measures in pregnant women.

CHAPTER 2

STUDY DESIGN AND METHODS

CHAPTER 2 – STUDY DESIGN AND METHODS

2.1 Study design and participants

The Be Healthy in Pregnancy (BHIP) study is an ongoing 2-arm randomized 3-site trial (www.clinicaltrials.gov NCT01689961) designed to assess the effect of a structured and monitored nutrition and exercise program (treatment) compared to standard prenatal care (control) on adherence to the IOM guidelines for GWG. Ethics approval was obtained from the Research Ethics Boards of Hamilton Health Sciences, Western University in London, and Joseph Brant Hospital in Burlington all in Southern Ontario, Canada. Healthy pregnant women were recruited from health care clinics in Hamilton, Burlington and London between 12 and 17 weeks gestation. Health care providers such as midwives and family physicians served as the first point of contact for recruitment by informing their clients/patients about the BHIP study. Consent to contact was obtained by completing a form containing participants' personal information, which was subsequently faxed to study staff or by contacting the study staff directly. Recruitment poster advertisements were also placed in participating hospitals and in the community and interested parties could phone or email the BHIP study staff directly. Following consent to contact, a scripted screening phone call was used to determine if the interested party is eligible to participate (see Table 2 for inclusion and exclusion criteria), to provide further information regarding the BHIP study and schedule the baseline (12-17 weeks gestation) study visit. Informed written consent was obtained from all participants at the baseline study visit. Randomization was stratified by study site and pBMI using a 24-hr centralized online randomization service managed by the Biostatistics Unit at

St. Joseph's Healthcare – Hamilton. Randomization occurred at the follow up visit, after all baseline data has been collected. As enrollment for the BHIP study was ongoing at the time of analysis, it was not possible to conduct analysis of data by treatment groups. The research that formed the basis of this thesis was thus an observational analysis of baseline measurements prior to randomization. Participants included in this analysis were a subset of the BHIP study sample with complete data sets available for all outcome measures: cardiometabolic profile, maternal adiposity, and dietary and physical activity data. Socio-demographic factors of the participants were assessed via questionnaires.

Table 2: Inclusion and exclusion criteria for the BHIP study

Inclusion Criteria	Exclusion Criteria
✓ Healthy pregnant females >18 years of age with singleton pregnancies (either nulliparous or multiparous) ✓ Less than 17 weeks' gestation ✓ Pre-pregnancy BMI <40 kg/m^2 ✓ Plans to deliver at a Hamilton or London regional hospital or by home birth ✓ Able to tolerate dairy foods ✓ Approval of primary care provider (as indicated by PARmed-X)[102] ✓ Able to provide signed informed consent	× Unable to understand some English × Type I or II diabetes × Known contraindications to exercise as recommended by Canadian clinical practice guidelines for pregnancy × Severe chronic gastrointestinal diseases or conditions × Refusal to consume dairy foods due to intolerance or dislike × Any significant heart, kidney, liver or pancreatic diseases × Currently smoking × A depression score above 12 on the validated Edinburgh depression questionnaire[103,104]

2.2 Anthropometric and body composition outcome measures

Height and weight were measured at the baseline visit. Height was measured using a wall-mounted statiometer (Ellard Instrumentation, Monroe WA). Current weight was determined using the Tanita® BF-350 Body Composition Analyzer (Arlington Heights, IL). Pre-pregnancy BMI was calculated using current height and pre-pregnancy weight quantified as current weight subtracted from self-reported weight gain. Skinfold thickness was measured by trained study staff in triplicate on the right-hand side of the body using the Harpenden skinfold caliper at four sites: subscapular, triceps, biceps, and suprailiac crest. Triplicate values at each site were averaged and summed to calculate SFT. SFT values were then inputted into body density and % body fat equations previously used in a pregnant population.[100]

$$SFT = \bar{x}\ (triceps) + \ \bar{x}\ (biceps) + \bar{x}\ (subscap) + \bar{x}\ (iliac\ crest)$$

$$body\ density = 1.1581 - 0.0720(SFT)$$

$$\%\ body\ fat = \left(\frac{4.95}{density} - 4.50\right) \times 100$$

In addition, percent body fat was recorded from BIA using the Tanita® BF-350 Body Composition Analyzer. A small electrical signal sent from four metal electrodes measured resistance through tissues in the feet and legs as participants stood on the scale. Body composition was calculated using validated equations proprietary to Tanita® by inputting resistance. Percent body fat calculated by both SFT and BIA characterized women into one of two categories: normal (<31%) or

overweight/obese (≥31%). Categories for % body fat were derived from data in a non-pregnant population as reference values specific to pregnancy do not exist.[105]

2.3 Dietary and physical activity assessment

Dietary intake was assessed at baseline by a standard three-day food intake record. Participants were asked to record everything they ate and drank for three consecutive days, including two weekdays and one weekend day. Nutritionist Pro™ Diet Analysis software, version 5.2.0 (Axxya Systems, Woodinville WA) was used to analyse the diet records for macro- and micronutrient intake. Participants also completed a PrimeScreen food frequency questionnaire (FFQ)[106] specially modified for the BHIP study (See Appendix 2). Currently, there is no widely accepted diet index used to score PrimeScreen. Thus, a novel diet index was created for the BHIP Study to assess diet quality during pregnancy which was based on a previous PrimeScreen dietary score method developed for non-pregnant populations.[107] *The Sensible Guide to a Healthy Pregnancy* published by the Ministry of Health was used as a reference for healthful dietary habits in pregnancy.[13] Each subscale of answers was dichotomized assigning a value of 1 to healthful frequencies of intake and 0 to unhealthy frequency of consumption (See Appendix 2 for scoring). An overall score from 0 (unhealthy dietary patterns) to 25 (healthier dietary patterns) was calculated for each participant.

Physical activity was assessed at baseline using the SenseWear® armband tri-axis accelerometer (Model MF-SW; BodyMedia® Inc., Pittsburgh PA). The device was worn for 72 consecutive hours that coincided with the three-day diet record.

Participants were instructed to wear the armband on the back of the upper left arm (i.e. triceps) for 24 hours a day except when showering, bathing, or swimming. The sensors in the device measure skin temperature, galvanic skin response, heat flux from the body, and movement. These data are then processed by propriety algorithms to calculate energy expenditure, step count, metabolic equivalents and sleep duration (SenseWear® Professional 8.1 Software; BodyMedia® Inc., Pittsburgh PA).

2.4 Laboratory procedures

Fasted venous blood samples were drawn at baseline with a total volume of 20mL split into four vials: sodium fluoride/ Na_2 ethylenediaminetetraacetic acid (EDTA) (1mL); PAXgene (3mL); SST™ Serum Separation Tubes with gel (5mL) and silicone coated (10mL). Samples were centrifuged for 10 minutes at 3000 rpm and 4°C; serum separator tubes spun for an additional 5 minutes, aliquoted, and stored in polypropylene microcentrifuge tubes at -20ºC for at least 24 hours before transfer to -80ºC.

2.4.1 Primary outcomes- glucose and triglycerides

Fasting plasma glucose was determined using a hexokinase photometric assay (Architect kit, Abbott, Abbott Park IL) completed by Hamilton Health Sciences Regional Laboratory Medicine Program (HRLMP). Glucose-6-phosphate (G-6-P) was produced following phosphorylation of glucose by hexokinase in the presence of adenosine triphosphate (ATP) and magnesium ions. G-6-P was then oxidized using glucose-6-phosphate dehydrogenase to form 6-phosphogluconate. Simultaneously, nicotinamide adenine dinucleotide (NAD) was reduced to nicotinamide adenine

dinucleotide reduced (NADH). For each micromole of glucose consumed, one micromole of NADH is produced. The quantity of NADH in the sample was detected spectrophotometrically as absorbed light at 340 nm (Abbott Architect *c*4000, Abbott Park, IL). The system was calibrated approximately every 30 days and the calibration curve ranged from 0.28 to 44.4 mM. Positive and negative quality control were run daily. The required sample volume was 100μL and assay coefficient of variation (CV) was ≤5%. Samples were initially tested neat with subsequent automatic dilution of 1:5 performed if values exceeded 44.4 mM. Expected values of plasma glucose in healthy pregnant women with normal BMI are detailed in Table 3.

Table 3: Reference ranges of cardiometabolic biomarkers in healthy pregnant women with normal BMI

Analyte	**Reference Range**	**Citations**
Glucose †	4 – 6 mM	Laboratory[108]
Insulin †	<25 μIU/L	Laboratory[109]
HOMA-IR	0.5 – 2 units	Derived from literature[110]
QUICKI	0.3-0.4 units	Derived from literature[111]
Triglycerides	40 – 159 mg/dL	Laboratory[35]
Leptin	<35 ng/mL	Derived from literature[112-114]
Adiponectin	6 – 21 μg/mL	Derived from literature[115,116]
CRP	<7 mg/L	Derived from literature[41,117]

†Reference ranges for non-pregnant women were used as values in early pregnancy do not different from pregravid.

Fasting serum triglycerides were analyzed using a glycerol phosphate oxidase photometric assay (Architect kit, Abbott, Abbott Park IL) at HRLMP. Briefly, triglycerides were enzymatically hydrolyzed to free fatty acids and glycerol via

lipase. Glycerol kinase (GK) with ATP was then used to phosphorylate glycerol to produce glycerol-3-phosphate and adenosine diphosphate. Hydrogen peroxide (H_2O_2) was produced following the oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate by glycerol phosphate oxidase. Next, a red coloured dye was produced following the reaction of H_2O_2 with 4-aminoantipyrine and 4-chlorophenol catalyzed by peroxidase. Triglyceride concentration in the sample was proportional to the absorbance of red coloured dye at 510 nm (Abbott Architect *c*8000, Abbott Park IL). The system was calibrated approximately every 41 days and the calibration curve ranged from 0 to 1420 mg/dL. Quality control was completed daily with a normal and abnormal control. The required sample volume was 100μL and assay CV was ≤5%. Samples were initially tested with neat dilution factor. Subsequent automatic dilution of 1:4 was performed if values exceeded 1420 mg/dL. Expected values of serum triglycerides in healthy pregnant women with normal BMI are detailed in Table 3.

2.4.2 Secondary outcomes- leptin, insulin, adiponectin and CRP

Serum leptin and insulin were analyzed using Luminex® human premixed multi-analyte enzyme-linked immunosorbent assay (ELISA) kit supplied by R&D Systems (Minneapolis MN). The required sample volume was 50μL and pooled plasma was used as an internal quality control. Fresh standards and reagents were prepared for each plate tested. The recommended dilution factor was 2-fold for serum samples in which 75 μL of sample was added to 75 μL of calibrator diluent. A test plate was run to determine the optimal dilution factor using neat, 1:2, 1:5, and 1:10

dilution of samples. Dilution factors were assessed by examining the CV between replicates and whether experimental values fell within the expected reference ranges (See Table 3). It was determined that a 1:2 dilution factor was most appropriate for the samples collected as part of this study. A total of 456 samples were analyzed using 12 plates. A pooled plasma sample was run in triplicate on each plate that yielded an intra-assay CV% of ≤9.1 and inter-assay CV% of ≤13.5.

Colour-coded magnetic microparticles pre-coated with analyte-specific antibodies were pipetted into wells followed by standards and samples. The analytes of interest are bound to the immobilized antibodies already present in the wells. The plate was then washed with wash buffer (100μL x 3 times) according to the protocol provided using a magnetic plate washer (Bio-Rad Bio-Plex Pro™ Wash Station) and human premixed biotin-antibody cocktail was added. Following a subsequent wash to remove any unbound biotin, streptavidin-phycoerythrin (Streptavidin-PE) conjugate was added to the wells and bound to the biotinylated antibody. Any unbound Streptavidin-PE was removed during the final wash cycle and the microparticles were re-suspended in surfactant buffer. The plate was read using the Bio-Rad Bio-Plex® 200. One light emitting diode (LED) classified the bead to determine the analyte that was detected while a second LED determined the amount of bound analyte present by measuring the magnitude of the Streptavidin-PE signal.

Serum adiponectin and CRP were also analyzed by Luminex® premixed multi-analyte ELISA kit supplied by R&D Systems using the aforementioned ELISA protocol except for differences in sample dilution factor. The suggested 200-fold

dilution was performed by adding 10 μL of sample to 90μL of calibrator diluent which was further diluted by adding 10μL of diluted sample to 190μL of calibrator diluent. Once more, dilution factor was confirmed after running a test plate using 1:100, 1:200, 1:500, and 1:1000 dilutions of samples. A total of 342 samples were analyzed over 9 plates, obtaining intra-assay CV% of ≤11.3 and inter-assay CV% of ≤10.7. Expected values of serum adiponectin and CRP in healthy pregnant women with normal BMI are detailed in Table 3.

2.5 Other measurements – insulin sensitivity

Insulin sensitivity was calculated using Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) equations previously determined using data from a non-pregnant population[118,119] and subsequently validated for use in a pregnant population.[120,121] Expected values of HOMA-IR and QUICKI in healthy pregnant women with normal BMI are detailed in Table 3.

$$HOMA\text{-}IR = \frac{[insulin \times glucose]}{22.5}$$

$$QUICKI = \frac{1}{[log(insulin) + log(glucose)]}$$

2.6 Statistical Analysis

Statistical analysis was performed using STATA 14 (StataCorp). 2015, Texas). Descriptive statistics were computed by calculating the mean and standard deviation if the

variable was normally distributed, or median and interquartile range (IQR) if the variable had non-normal distribution.

McNemar's tests were used to assess whether there was a difference in the proportion of participants being categorized as normal or overweight/obese by pBMI, and % body fat estimates from BIA or SFT measurements. The agreement between % body fat from BIA or SFT was evaluated using a Bland-Altman plot created using GraphPad Prism 7.03 (GraphPad Software, Inc., La Jolla CA).

Physical, dietary and PA characteristics were compared between women categorized as normal vs. overweight/obese (by SFT or BIA) using chi-square tests for categorical factors, t-tests for continuous normally distributed variables, and Mann-Whitney U tests for continuous variables with non-normal distributions.

Linear regression was used to determine whether cardiometabolic biomarkers were correlated with maternal adiposity (i.e. % body fat from BIA or SFT). Cardiometabolic biomarkers (i.e. insulin, triglycerides, leptin, adiponectin, CRP) were natural log transformed, except for glucose, when shown to be of non-normal distribution. Multiple linear regression was conducted to adjust the relationship between body adiposity and cardiometabolic biomarkers for potential confounding variables related to demographics, dietary and physical activity factors using the appropriate data transformation (Table 4). Akaike information criterion values were computed for all unadjusted and adjusted models to compare the strength of association of maternal adiposity on cardiometabolic profiles between SFT and BIA measures.[122]

Table 4: List of confounding variables included in multiple regression models – variable type and data transformations described

Variable	Type	Transformation
Age	Categorical	None
Ethnicity	Categorical	None
Parity	Categorical	None
Energy expenditure (kcal)	Continuous	Logarithmic
Time spent at moderate to vigorous activity (minutes)	Continuous	Square root
Total energy intake (kcal)	Continuous	None
Polyunsaturated: saturated fat ratio	Continuous	Square root

CHAPTER 3

RESULTS

CHAPTER 3 – RESULTS

Section A: Evaluation of adiposity measurements

A.3.1 Maternal characteristics and physical measures

The demographic characteristics of participants (n=213) that had available measurements of % body fat data from both BIA and SFT at baseline and pBMI $\geq$18.5kg/m^2 are presented in Table 5. The majority of women were well educated, married, and had an annual household income above $75,000. Nulliparous women comprised nearly half of the study group.

The physical measures of participants are summarized in Table 6. Over 50% of women were categorized as having normal pBMI with the remainder categorized as overweight or obese. Underweight women (n=4) were excluded from the study group as categorization by body fat does not exist for individuals with BMI <18.5 kg/m2.

Table 5: Demographic characteristics of participants included in adiposity measurement analysis

Demographic factors	**N**	**%**
Age at enrollment (years)	31 ± 4	
Gestational stage at enrollment (weeks)	13 (2)	
Education level	213	-
High school/some college/university	9	4.2
College/trade school certificate or diploma	35	16.4
Bachelor's degree	67	31.5
Above Bachelor's degree	101	47.4
Other	1	0.5
Household income (per year)	213	-
<$30,000	8	3.8
≥$30,000 to <$75,000	48	22.5
≥$75,000	148	69.5
Prefer not to answer/don't know	9	4.2
Marital status	211	-
Married/Common law/living with partner	205	97.2
Single	6	2.8
Ethnicity	213	-
Caucasian	184	86.4
Other	29	13.6
Parity	212	-
Nulliparous (0 pregnancies)	103	48.6
Primiparous (1 pregnancy)	61	28.8
Multiparous (≥2 pregnancies)	48	22.6

[1]Values reported as Mean ± SD unless otherwise indicated
[2]Values reported as Median with IQRs in parentheses if not normally distributed

Table 6: Physical measures of participants included in adiposity measurement analysis

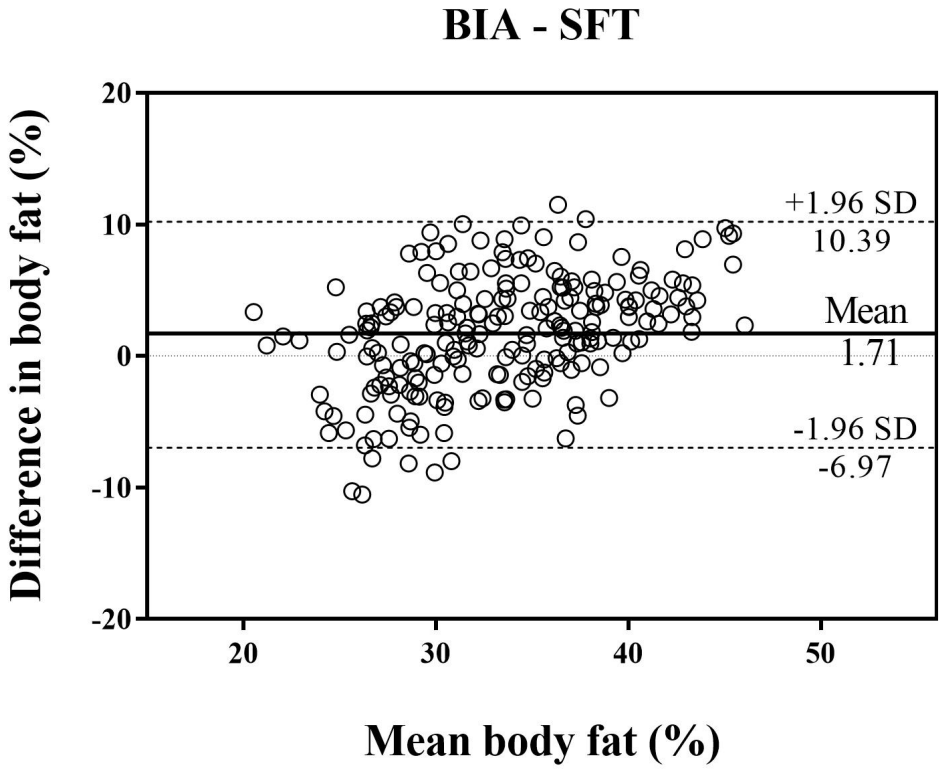
Physical measures	**N**	**%**
Pre-pregnancy BMI	213	-
Normal weight (18.5 – 24.9 kg/m^2)	116	54.5
Overweight (25.0 – 29.9 kg/m^2)	60	28.2
Obese (≥30.0 kg/m^2)	37	17.4
Weight (kg)	70.9 (16.4)	
Height (m)	1.65 ± 0.06	
SFT (mm)	67 (35)	
Percent body fat (SFT)	32.6 ± 4.9	
Percent body fat (BIA)	34.3 ± 6.7	

[1]Values reported as Mean ± SD unless otherwise indicated
[2]Values reported as Median with IQRs in parentheses if not normally distributed

A.3.2 Comparison of percent body fat between methods

Bioelectrical impedance analysis yielded % body fat measurements that were 1.71% greater compared to those estimated using SFT (n=213, r=0.44, p<0.001) (Figure 2). The 95% limits of agreement were 10.39 and -6.97.

Figure 2: Bland-Altman plot of mean vs. difference of body fat (%) from BIA compared to SFT. Percent body fat was 1.71% greater when using BIA compared to SFT. (n=213, r=0.44, p<0.001)



A.3.3 Comparison of normal vs. overweight/obese categorization by body fat

The proportion of participants categorized as overweight/obese by % body fat by BIA was greater than by SFT measures (67.6% vs. 58.7%) (Table 7). When participants were categorized by pBMI, the proportion categorized as overweight/obese was significantly lower ($p<0.0001$) when compared to either SFT (Table 8) or BIA (Table 9). Pre-pregnancy BMI underestimated FM in 16.9% of the sample when percent body fat was estimated from SFT, and in 23.0% when estimated from BIA.

Table 7: Comparison of normal (<31% body fat) and overweight/obese (≥31% body fat) participants defined by BIA and SFT. McNemar's test determined a significant difference in the proportion of women being categorized as normal and overweight/obese. ($p<0.01$)

		SFT	
		Normal	Overweight/Obese
BIA	Normal	55	14
	Overweight/Obese	33	111

Table 8: Comparison of normal (<31% body fat) and overweight/obese (≥31% body fat) categorization defined by SFT and pBMI. McNemar's test determined a significant difference in the proportion of women being categorized as normal and overweight/obese. ($p<0.0001$)

		SFT	
		Normal	Overweight/Obese
pBMI	Normal	80	36
	Overweight/Obese	8	89

Table 9: Comparison of normal (<31% body fat) and overweight/obese (≥31% body fat) categorization defined by BIA and pBMI. McNemar's test determined a significant difference in the proportion of women being categorized as normal and overweight/obese. ($p<0.0001$)

		BIA	
		Normal	Overweight/Obese
pBMI	Normal	67	49
	Overweight/Obese	2	95

Section B: Clinical study

B.3.1 Maternal characteristics and physical measures

A total of 91 women with singleton pregnancies had complete data available for most cardiometabolic biomarkers, maternal adiposity, and dietary and physical activity data in early pregnancy. Sample sizes for adiponectin (n=72) and CRP (n=67) were smaller as laboratory analyses were not completed for these biomarkers in all participants. Lipid and glucose profiles of the other biomarkers were measured in 141 participants with corresponding adiposity data. Dietary and PA data were missing for a total of 48 participants reducing the sample size (n=93). Data were also excluded for the following situations: one extreme value for energy expenditure (7500 kcal/d) was affecting the normality of the data sets even after transformation was performed as all other values were <3004 kcal/d; two extreme values of polyunsaturated: saturated fat (P:S) ratio were excluded due to normality issues following transformation.

Demographic characteristics of all participants are summarized in Table 10. The majority of the participants were well educated, married and had an annual household income of greater than $75,000. Nearly half of the sample was comprised of nulliparous women.

Table 10: Demographic characteristics of participants (n=91) included in regression analysis

Demographic factors	**N**	**%**
Age at enrollment (years)[1]	31 ± 4	
Gestational stage at enrollment (weeks)[2]	13 (2)	
Education level	91	-
College/trade school certificate or diploma	20	21.9
Bachelor's degree	27	29.7
Above Bachelor's degree	43	47.3
Other	1	1.1
Household income	91	-
<$30,000	3	3.3
≥$30,000 to <$75,000	19	20.9
≥$75,000	66	72.5
Prefer not to answer/don't know	3	3.3
Marital status	89	-
Married/Common law/living with partner	85	95.5
Single	4	4.5
Ethnicity	91	-
Caucasian	82	90.1
Other	9	9.9
Parity	91	-
Nulliparous (0 pregnancies)	41	45.1
Primiparous (1 pregnancy)	30	32.9
Multiparous (≥2 pregnancies)	20	22.0

[1]Values reported as Mean ± SD unless otherwise indicated
[2]Values reported as Median with IQRs in parentheses if not normally distributed

Physical measures of the participants on entry into the study are presented in Table 11. Over 50% of the women were categorized as normal pBMI with the remainder categorized overweight or obese. Underweight women (n=2) were excluded from the study group as categorization by body fat does not exist for women with BMI <18.5 kg/m^2.

Table 11: Physical measures of participants (n=91) included in regression analysis

Physical measures	**N**	**%**
Pre-pregnancy BMI	91	-
Normal weight (18.5 – 24.9kg/m^2)	49	53.8
Overweight (25.0 – 29.9kg/m^2)	26	28.6
Obese (≥30.0kg/m^2)	16	17.6
Height (m)	1.66 ± 0.07	
Weight (kg)	71.0 (20.9)	
SFT (mm)	61 (35)	
Percent body fat (SFT)	30.9 (7.7)	
Percent body fat (BIA)	34.5 ± 6.8	

[1]Values reported as Mean ± SD unless otherwise indicated
[2]Values reported as Median with IQRs in parentheses if not normally distributed

Dietary and PA characteristics are presented in Table 12. Average total energy intake (2106 kcal) was not significantly different from average energy expenditure (2069 kcal).

Table 12: Diet, energy expenditure and physical activity measures at study entry

	Total group (N=91)
Diet	
Total energy intake (kcal/day)	2106 ± 515
Polyunsaturated: saturated fat ratio	0.38 (0.24)
Accelerometry measures	
Energy expenditure (kcal/day)	2068 (469)
Time spent at moderate to vigorous activity (minutes/day)	49 (44)

[1]Values reported as Mean ± SD unless otherwise indicated
[2]Values reported as Median with IQRs in parentheses if not normally distributed

B.3.2 Cardiometabolic profiles

Cardiometabolic biomarker profiles of the women at study entry are presented in Table 13. The values for the cardiometabolic biomarkers were within the expected range for healthy pregnant women with normal BMI with a few exceptions. Fasting insulin and triglycerides were above the range for 3.3% and 8.8% of the sample, respectively. Values for adiponectin were below the expected range in 6.6% of the participants. For leptin 37.4% of participants had leptin values above the expected range and CRP in 23.1% of participants. HOMA-IR and QUICKI values were outside the expected range for healthy pregnant women in 18.7% and 3.3% of the participants, respectively.

Table 13: Cardiometabolic biomarker profiles of participants at study entry with cut-off values noted that were used to define outside the expected range for healthy pregnant women of normal pBMI

Cardiometabolic markers	**N**	**Participants Median (IQR)**	**Outside expected range**
Glucose (mM)	91	4.8 ± 0.5	>6 [108]
Insulin (μIU/L)	91	4.8 (4.0)	>25.0 [109]
HOMA-IR	91	1.0 (0.97)	<2 [110]
QUICKI	91	0.38 ± 0.05	>0.3 [111]
Triglycerides (mg/dL)	91	104.4 (51.3)	>159 [35]
Leptin (ng/mL)	91	21.7 (28.8)	>35 [112-114]
Adiponectin (μg/mL)	72	10.6 (3.4)	<6 [115,116]
C-reactive protein (mg/L)	67	5.2 (5.6)	>7 [41,117]

[1]All values computed using untransformed data
[2]Values reported as Mean ± SD unless otherwise indicated
[3]Values reported as Median with IQRs in parentheses if not normally distributed

B.3.3 Comparison of maternal characteristics by subject adiposity category

Maternal characteristics were compared between women categorized as normal or overweight/obese defined by percent body fat estimated from SFT measurements in Table 14. Demographic and dietary factors were not significantly different between the two groups. For physical measurements, overweight/obese women were heavier ($p<0.0001$) and had greater SFT ($p<0.0001$) and body fat ($p<0.0001$) compared to those categorized as normal. Energy expenditure was higher in the overweight/obese group, while MVPA was lower compared to women with normal percent body fat.

Table 14: Comparison of maternal characteristics between normal and obese participants defined by % body fat estimates from 4-site skin-fold thickness (SFT)

Maternal characteristics	Normal (n=47)	Overweight/ Obese (n=44)	P value
Demographic			
Age (years)	31 ± 1	31 ± 1	ns
Ethnicity[1]	-	-	ns
Parity[1]	-	-	ns
Physical measures			
Height (m)	1.67 ± 0.01	1.65 ± 0.01	ns
Weight (kg)	62.6 (12.4)	82.5 (18.3)	<0.0001
SFT (mm)	52 (13)	87 (33)	<0.0001
Percent body fat (SFT)	28.6 (3.8)	36.1 (5.6)	<0.0001
Dietary factors			
PrimeScreen healthy eating score[†]	9.2 ± 0.4	8.8 ± 0.5	ns
Total energy intake (kcal)	2070 ± 76	2144 ± 77	ns
Carbohydrate (% energy)	51.5 ± 1.1	50.9 ± 1.1	ns
Protein (% energy)	15.5 (3.3)	14.9 (3.3)	ns
Fat, total (% energy)	32.7 ± 1.0	33.4 ± 0.8	ns
Polyunsaturated: saturated fat ratio	0.34 (0.27)	0.39 (0.20)	ns
Accelerometry measures			
Energy expenditure (kcal/d)	1999 (279)	2279 (522)	<0.001
Time spent at moderate to vigorous intensity level (minutes)	55 (51)	40 (35)	<0.02

[1]Categorical variables (ethnicity: Caucasian or other; parity: nulliparous, primiparous or multiparous)
[2]Values reported as Mean ± SD unless otherwise indicated
[3]Values reported as Median with IQRs in parentheses if not normally distributed
[†]N=33 per group

When categorized using % body fat measured by BIA (Table 15), the overweight/obese women were significantly older than those in the normal group. Measures of maternal adiposity remained greater in the overweight/obese group. However, time spent at moderate to vigorous intensity level was no longer significantly different between the two groups.

Table 15: Comparison of maternal characteristics between normal and obese participants defined by % body fat measured using bioelectrical impedance analysis (BIA)

Maternal characteristics	Normal (n=30)	Overweight/ Obese (n=61)	P value
Demographic			
Age (years)	30.2 ± 0.8	31.9 ± 0.4	<0.05
Ethnicity[1]	-	-	ns
Parity[1]	-	-	ns
Physical measures			
Height (m)	1.64 ± 0.01	1.67 ± 0.01	ns
Weight (kg)	60.9 (5.9)	78.9 (14.2)	<0.0001
SFT (mm)	51 (13)	73 (38)	<0.0001
Percent body fat (BIA)	26.9 ± 2.5	38.3 ± 4.7	<0.0001
Dietary factors			
PrimeScreen healthy eating score[2]	9.0 ± 0.5	9.0 ± 0.4	ns
Total energy intake (kcal)	1990.6 ± 93.3	2162.8 ± 65.6	ns
Carbohydrate (% energy)	50.6 ± 1.3	51.5 ± 0.9	ns
Protein (% energy)	16.3 (3.5)	14.9 (2.7)	ns
Fat, total (% energy)	33.4 ± 1.2	32.9 ± 0.7	ns
Polyunsaturated: saturated fat ratio	0.42 (0.31)	0.36 (0.22)	ns
Accelerometry measures			
Energy expenditure (kcal/d)	1962 (278.0)	2228 (489)	<0.0001
Time spent at moderate to vigorous intensity level (minutes)	52 (45)	45 (43)	ns

[1]Categorical variables (ethnicity: Caucasian or other; parity: nulliparous, primiparous or multiparous)
[2]Values reported as Mean ± SD unless otherwise indicated
[3]Values reported as Median with IQRs in parentheses if not normally distributed
[†]N= 22 for normal and n=44 for overweight/obese group

B.3.4 Association between maternal characteristics and cardiometabolic status by multiple linear regression analysis

B.3.4.1 Adiposity by sum of skinfold thickness

Regression models were carried out using data from the study group (n=91) described in sections B.3.1 – B.3.2. In early pregnancy, percent body fat estimated from SFT was significantly associated with higher concentrations of glucose, insulin, triglycerides, leptin, and CRP, and lower adiponectin (Table 16). The associations remained statistically significant, albeit slightly attenuated, after adjustment for known confounding factors related to demographic, dietary, and physical activity measures (Table 16). The exception was CRP in which the adjusted model did not fit the data (n=67, F (9, 57) =2.03, p=0.052, r^2=0.243). The strength of association of % body fat from SFT on cardiometabolic status is presented in Table 17. Maternal adiposity was predominantly associated with leptin, insulin, and glucose concentrations in early pregnancy as demonstrated by their relatively large R^2 and significant p values in both the unadjusted and adjusted models.

Table 16: Linear regression analyses of maternal adiposity (% body fat from 4-site measures of SFT) and its association with cardiometabolic biomarkers

Outcomes	N	Unadjusted			Adjusted		
		β	95% CI	P value	β	95% CI	P value
Glucose (mM)	91	1.51	(0.99, 2.03)	<0.001	1.11	(0.47, 1.74)	<0.01
Insulin (μIU/L) †	91	2.69	(1.94, 3.44)	<0.001	1.93	(1.02, 2.83)	<0.001
Triglycerides (mg/dL) †	91	1.11	(0.65, 1.57)	<0.001	0.87	(0.28, 1.47)	<0.01
Leptin (ng/mL) †	91	3.88	(3.10, 4.66)	<0.001	3.33	(2.39, 4.26)	<0.001
Adiponectin (μg/mL) †	72	-1.02	(-1.47, -0.57)	<0.001	-0.81	(-1.38, -0.25)	<0.01
C-reactive protein (mg/L) †	67	2.21	(0.97, 3.45)	<0.01	-	-	-

[1]Multiple linear regression was performed for each outcome, adjusting for: age (years), ethnicity, parity, energy expenditure (kcal/d), moderate-to-vigorous physical activity (min/d), total energy intake (kcal/d), polyunsaturated: saturated fat ratio
† Values natural log transformed prior to regression analysis

Table 17: Goodness of fit statistics for linear regression analyses of maternal adiposity (% body fat from 4-site measures of SFT) and its association with cardiometabolic biomarkers. Body fat percentage was predominantly associated with leptin, insulin, and glucose status in early pregnancy

Outcomes	**N**	**Unadjusted**			**Adjusted**		
		R^2	**P value**	**AIC**	**R^2**	**P value**	**AIC**
Glucose (mM)	91	0.27	<0.0001	89.0	0.40	<0.0001	87.3
Insulin (μIU/L) †	91	0.37	<0.0001	154.7	0.49	<0.0001	150.9
Triglycerides (mg/dL) †	91	0.20	<0.0001	66.8	0.28	<0.01	74.3
Leptin (ng/mL) †	91	0.52	<0.0001	162.5	0.63	<0.0001	156.5
Adiponectin (μg/mL) †	72	0.23	<0.0001	34.6	0.38	<0.001	35.3
C-reactive protein (mg/L) †	67	0.16	<0.001	162.1	-	-	-

[1]Multiple linear regression was performed for each outcome, adjusting for: age (years), ethnicity, parity, energy expenditure (kcal/d), moderate-to-vigorous physical activity (min/d), total energy intake (kcal/d), polyunsaturated: saturated fat ratio
† Values natural log transformed prior to regression analysis

Some of the confounding variables in the adjusted models were also significantly associated with cardiometabolic biomarker status in early pregnancy (Appendix 3). Fasting glucose was positively associated with P:S ratio (β=0.90, $p<0.01$). Fasting insulin was positively associated with energy expenditure (β=1.78, $p<0.01$) and negatively associated with age (β=-0.38, $p<0.05$) and MVPA (β=-0.06, $p<0.05$). Triglycerides were significantly higher in non-Caucasian women compared to Caucasian women with the same % body fat (β=0.27, $p<0.05$). Leptin was positively associated with energy expenditure (β=1.48, $p<0.01$) and negatively associated with P:S ratio (β=-0.94, $p<0.05$). Adiponectin was significantly higher in multiparous women compared to nulliparous women with the same % body fat (β=-0.29, $p<0.01$).

B.3.4.2 Bioelectrical impedance analysis

Regression models were repeated using percent body fat measured by BIA. Similar to the previous analysis using SFT, percent body fat was significantly associated with the cardiometabolic biomarkers in early pregnancy (Table 18). Following adjustment for demographic, dietary, and physical activity factors, these associations remained significant albeit slightly attenuated except for maternal adiponectin which was no longer significantly associated (Table 18). CRP data also did not fit the adjusted model (n=67, $F(9, 57) = 1.01$, $p=0.446$, $r^2=0.137$). However, the strength of the associations in both the unadjusted and adjusted BIA models was weaker than for the SFT models (Table 17) as demonstrated by smaller R^2 and AIC values in the BIA models (Table 19). Maternal

adiposity remained predominantly associated with leptin, insulin, and glucose profiles in early pregnancy.

Table 18: Linear regression analyses of maternal adiposity (% body fat from BIA) and its association with cardiometabolic biomarkers

Outcomes	N	Unadjusted			Adjusted		
		β	95% CI	P value	β	95% CI	P value
Glucose (mM)	91	0.04	(0.02, 0.05)	<0.001	0.03	(0.01, 0.05)	<0.01
Insulin (μIU/L) †	91	0.05	(0.04, 0.07)	<0.001	0.03	(0.01, 0.06)	<0.05
Triglycerides (mg/dL) †	91	0.02	(0.01, 0.03)	<0.001	0.02	(0.01, 0.04)	<0.05
Leptin (ng/mL) †	91	0.08	(0.06, 0.10)	<0.001	0.08	(0.04, 0.11)	<0.001
Adiponectin (μg/mL) †	72	-0.02	(-0.03, -0.01)	<0.001	-0.02	(-0.03, 0.01)	ns
C-reactive protein (mg/L) †	67	0.04	(0.01, 0.07)	<0.05	-	-	-

[1]Multiple linear regression was performed for each outcome, adjusting for: age (years), ethnicity, parity, energy expenditure (kcal/d), moderate-to-vigorous physical activity (min/d), total energy intake (kcal/d), polyunsaturated: saturated fat ratio
† Values natural log transformed prior to regression analysis

Table 19: Goodness of fit statistics for linear regression analyses of maternal adiposity (% body fat from 4-site measures of SFT) and its association with cardiometabolic biomarkers. Body fat percentage remained predominantly associated with leptin, insulin, and glucose status in early pregnancy

Outcomes	**N**	**Unadjusted**			**Adjusted**		
		R^2	**P value**	**AIC**	**R^2**	**P value**	**AIC**
Glucose (mM)	91	0.28	<0.0001	88.0	0.40	<0.0001	88.0
Insulin (μIU/L) †	91	0.27	<0.0001	167.4	0.41	<0.0001	163.5
Triglycerides (mg/dL) †	91	0.17	<0.001	71.0	0.25	<0.01	77.7
Leptin (ng/mL) †	91	0.43	<0.0001	178.1	0.52	<0.0001	178.6
Adiponectin (μg/mL) †	72	0.16	<0.001	40.9	0.33	<0.01	40.6
C-reactive protein (mg/L) †	67	0.07	<0.05	169.1	-	-	-

[1]Multiple linear regression was performed for each outcome, adjusting for: age (years), ethnicity, parity, energy expenditure (kcal/d), moderate-to-vigorous physical activity (min/d), total energy intake (kcal/d), polyunsaturated: saturated fat ratio
† Values natural log transformed prior to regression analysis

Differences existed regarding the significant confounding factors between the adjusted BIA and SFT models (See Appendix 3). Maternal leptin concentration was negatively associated with age (β=-0.05, $p<0.05$) rather than positively associated with energy expenditure and negatively associated with P:S ratio as observed in the SFT regression model. Furthermore, multiparity (β=-0.29, $p<0.01$) was the only independent variable significantly associated with lower adiponectin concentration in early gestation whereas body fat was no longer significantly associated with adiponectin concentration unlike in the SFT model.

Chapter 4

DISCUSSION & FUTURE DIRECTIONS

CHAPTER 4 – DISCUSSION & FUTURE DIRECTIONS

4.1 Maternal characteristics associated with cardiometabolic profiles in early pregnancy

Pregravid overweight and obesity increase obstetric risk during pregnancy and have been associated with short and long term adverse health consequences for both women and their offspring.[14] In our investigation of cardiometabolic indicators of health risks in early pregnancy we observed that quantitative measures of % body fat were found to significantly associated with all six cardiometabolic biomarkers although predominantly for leptin, glucose and insulin. Adjusting for confounding factors related to demographics, diet and physical activity only slightly attenuated the associations.

While both methods (i.e. SFT and BIA) to quantitate body fat were similarly associated with cardiometabolic status, SFT measures produced a slightly stronger association in both the unadjusted and adjusted models. We also observed that adiponectin was significantly associated with % body fat estimated from SFT but not by BIA. Previously, BIA measures were found to be negatively associated with maternal adiponectin status in pregnancy. [64,67] The diverse findings might be explained by differences in the technique used between studies for BIA measures. While our study employed a leg-to-leg method of BIA which mainly measured thigh adiposity, the prior studies used the tetrapolar (i.e. hand-to-foot) method which measures adiposity throughout the body including the abdominal region. In the case of the significant association of adiponectin with SFT, this measure includes a skinfold thickness measure located in the right iliac region of the abdomen. This might suggest that abdominal

adiposity has a greater influence on adiponectin status in pregnant women as studies conducted in non-pregnant populations have demonstrated.[123]

Screening for maternal obesity and related cardiometabolic dysfunction may be more beneficial when targeted to certain groups of pregnant women. Our study revealed that some demographic variables were significantly associated with cardiometabolic biomarker status in early pregnancy. Fasting insulin significantly declined with increasing maternal age. A previous study in non-pregnant adults also found an inverse association between age and insulin concentrations in women.[124]

Adiponectin was impacted by parity being lower in multiparous women compared to women who were nulliparous in early pregnancy. This appears to be a novel finding but is biologically plausible as adiponectin concentration is negatively associated with abdominal adiposity in non-pregnant populations[123] and parous women have higher abdominal adiposity than nulliparous women.[125] In the one study in pregnant populations, no significant relationship was noted between maternal adiponectin and abdominal subcutaneous and visceral FM in late pregnancy but it had a small sample size (n=20) and did not account for potential confounders such as parity.[45]

A trend of higher maternal triglycerides was observed in non-Caucasian women (i.e. Asian, Hispanic, First Nations) compared to Caucasian women with equal body fat percentage in early pregnancy. Our study group consisted of few non-Caucasian women, thus, definitive conclusions regarding this association cannot be made. While higher circulating triglycerides were previously observed in Asian and Hispanic pregnant women

compared to those of Caucasian descent, [84,126,127] differences in First Nation populations have not been reported.

Our study reinforces the importance of considering maternal characteristics such as age, parity, and ethnicity when screening for obesity in early pregnancy because cardiometabolic dysfunction related to excess adiposity may be further exacerbated in pregnant women who are younger, multiparous, or non-Caucasian. Consequently, their offspring may be at even higher risk for adverse health outcomes. Cardiometabolic biomarkers play an essential role in fetal growth and development by regulating placental nutrient transport and nutrient allocation to the fetus. Such observations underpin the link between abnormal maternal metabolic status in pregnancy and infant adiposity.[20] The incidence of adverse birth outcomes such as macrosomia and LGA are greater in multiparous women and those of Asian or Indigenous ethnic origins.[129-132] Interestingly, increasing maternal age at childbirth is associated with reduced abdominal fat and favourable cardiometabolic phenotype in children.[133] This suggests that maternal age has a protective effect against excess placental nutrient transport and fetal overgrowth linked to maternal insulin status.

This study may also provide guidance for future development of dietary and PA interventions aimed at improving maternal cardiometabolic function in pregnancy and reducing risk of adverse health outcomes in mothers and offspring. Regarding dietary fat intake, our findings cannot confirm previous observations[75] that a relationship exists between leptin and total dietary fat intake in early pregnancy. However, fat quality indicated by the P:S ratio was associated with lower maternal leptin. Dietary interventions

focused on reduced intake of saturated fats and increased polyunsaturated fats should be explored to determine if they provide beneficial effects to adipokine status in pregnancy and related infant outcomes.

The impact of PA level was only noted to be significantly associated with fasting insulin in early pregnancy. Our findings concur with previous evidence that PA intervention during gestation improves maternal insulin status in pregnancy.[70]

4.2 Evaluation of adiposity measurements

4.2.1 Comparison of percent body fat estimates

While our study indicated that SFT and BIA had similar strength of association with cardiometabolic status in early pregnancy, there were some quantifiable differences in comparing measures by the two methods. First, percent body fat estimated by BIA yielded values that were significantly higher and wider in range compared to SFT measures entered into a published equation to estimate % body fat. This translated into a greater number of women being categorized as overweight/obese by BIA than SFT.

The difference in % body fat between the two measures may relate to the difference in body compartments being measured. Preferential deposition of maternal fat accretion in the hips and thighs during the first six months of pregnancy may explain higher % body fat measures obtained by BIA.[134] Our study used a single frequency leg-to-leg analyzer to obtain BIA measurements; thus, the values largely represented FM in the thighs. Conversely, skinfold thickness measurements entered into the % body fat equation were primarily obtained from the arm and shoulder regions (i.e. biceps, triceps, subscapular), in addition to one measure in the abdominal area. Whether the leg-to-leg

method of BIA that captured the hip and thigh fat deposition that is characteristic of pregnancy is a more accurate measure of FM in pregnancy than the SFT estimates as applied in this study will have to be assessed by comparison to a gold-standard method such as DXA.

Application of our findings on body fat measures in the clinical setting needs to be considered from several perspectives. Estimation bias may explain the wide variation in % body fat measures by BIA compared to SFT. Bioelectrical impedance tends to underestimate at lower FM and overestimate at higher FM. It is a measure of TBW, which is then inputted into algorithms to sequentially estimate fat-free mass (FFM) and body fat, respectively.[113] In pregnancy, FFM density progressively declines resulting in underestimation of FFM and overestimation of FM.[135] Algorithms to convert impedance measures to % body fat specific for pregnant populations do not exist; thus, bias related to the physiological changes of pregnancy raises some concerns regarding its reliability. In obese individuals, a key assumption of BIA that the body acts like a cylindrical conductor is not valid.[136] Furthermore, obesity may alter hydration status prior to pregnancy.[136] A previous study by Marshall *et al*. compared multiple methods of measuring maternal adiposity in late pregnancy and found that overestimation occurred with increasing FM.[112] These biases extend the range of body fat values obtained by BIA. Future research should seek to advance BIA technology by developing novel algorithms that adjust for hydration changes observed at various stages of gestation.

Estimation bias also exist for SFT measurements. A tendency for overestimation of FM in leaner non-pregnant individuals and underestimation in obese persons has been

reported.[137] In obese women, % body fat tends to be underestimated due to difficulties in grasping the entire fold of skin.[137] As is the case with BIA, alterations in hydration status related to gestation may contribute to overestimations in % body fat estimated from SFT; however, findings are contradictory.[138-140] Extracellular and intracellular water increases as well as distortion of skin contour during pregnancy may lead to bias in skinfold thickness.[141] The equations that were selected to estimate % body fat from SFT measurements did not take into consideration such changes in TBW and skin contour in pregnant women. However, equations specific to pregnant populations did not exist to match the 4 skinfold sites measured in this study. Marshall *et al*. previously employed the same equations to estimate % body fat from SFT measures in late pregnancy and reported that the values obtained were comparable with % body fat measured by DXA in the early postpartum period.[112] Thus, we deemed that the use of these equations in a pregnant population is satisfactory.

From a practical perspective in the clinic setting, the replacement of pBMI with BIA may be easier to implement than SFT. Percent body fat measures are faster to obtain with BIA compared to SFT. The length of time required to conduct both methods of measurement was previously assessed in our lab (n=14) of BHIP participants. The mean time to collect BIA measures including set up time was 0.51 minutes compared to 5.15 minutes for SFT (See Appendix 4). Furthermore, body fat percentage is automatically computed by the BIA analyzer whereas SFT measures must be entered into equations to estimate % body fat thus further increasing the time to obtain measures of maternal adiposity. Bioelectrical impedance also requires fewer technical skills by the operator.[142]

Improper training for the technique of conducting skinfold measurements can increase inter-observer variation leading to bias.

4.2.2 Shortcomings of pre-pregnancy body mass index

Pre-pregnancy BMI is often used as a surrogate measure of maternal adiposity in research and clinical practice, however, it cannot distinguish body composition.[112] Further, the use of pBMI as a screening tool may not identify a number of at-risk pregnancies related to obesity. In quantifying the misclassification of adiposity status by pBMI compared to body fat measures taken in the beginning of the second trimester, we observed that pBMI underestimated adiposity in 16.9% of pregnant women when % body fat was estimated by SFT, and 23.0% by BIA.

4.2.3 Summary – The ideal method for obesity screening in early pregnancy

Our data suggest that screening of women for cardiometabolic risk by % body fat can be reliably achieved using either SFT or BIA, this despite the observation that BIA produces slightly higher values for % body fat. However, both methods identify a greater proportion of women in the overweight/obese category compared to just BMI alone. Taken together, from a practical application point of view, BIA may be the more ideal method for measuring maternal adiposity as a screening tool for cardiometabolic dysfunction in early pregnancy. First, the use of BIA on a large scale is practical because it is fast, user friendly, and inexpensive. Second, BIA is well tolerated by subjects and less invasive than skinfold thickness measurements. Lastly, leg-to-leg BIA may capture adiposity changes in early pregnancy better than SFT.

4.3 Strengths and limitations

Our study has several strengths. The numerous cardiometabolic biomarkers assessed in the current study permitted a more holistic analysis of the associations with maternal adiposity, dietary and PA factors. Dietary variables were measured objectively by three-day food intake record. Likewise, PA was objectively measured by accelerometry. Previous studies have reported overestimation of PA level by self-reported questionnaires in pregnant women.[143,144]

The methodologies chosen to measure maternal FM in early pregnancy also strengthen our study. Fat mass was quantitatively measured using SFT and BIA rather than simple physical measures such as BMI. Furthermore, BIA measurements were collected using the leg-to-leg method which is optimal for pregnancy. Unlike hand-to-foot methods, the electrical signal does not travel past the legs which prevents bias as the TBW contributions of the fetal and placental units are not included in the measurement.[145] The large sample size relative to previous studies evaluating methods of measuring fat mass enabled a more robust analysis of data.

Our study also had several limitations. Causal relationships cannot be determined as we performed an observational analysis of the data. Definitive inferences regarding the comparison of maternal adiposity measures could not be made as multiple McNemar's tests were chosen for the statistical analysis which can only be used to generate hypotheses.

From the perspective of measurement tools, no gold standard method of measuring adiposity in pregnancy was available to compare with SFT and BIA measures.

Both methods are indirect quantitative measures of body fat percentage and tend to underestimate % body fat compared to DXA. Further, the algorithms/equations used to calculate % body fat from BIA and SFT do not take into consideration the physiological adaptations related to pregnancy. Categorization by % body fat may not be accurate for a pregnant population because references were defined by data in a non-pregnant population.

The laboratory analytical methods for insulin had some limitations. For measures of insulin sensitivity, the blood samples were not collected in duplicate over a 10-minute period which is recommended by Mather *et al*.[146] Thus, biological variability in insulin due to its short half-life in serum, cyclical secretion, and prompt responsiveness to alterations in the hormonal and metabolic milieu was not considered; pregnancy may further exacerbate this variability. Quantifiable differences were observed regarding insulin sensitivity between log(HOMA-IR) and QUICKI. Both methods are preferable to other measures of insulin sensitivity as normality of distribution, variation, and repeatability is better.[146] Lack of consensus on the cut-off values for insulin resistance in pregnant populations limits our ability to definitively assess which of these two methods is preferable.

Recruitment location and demographics of the participant group studied may reduce the generalizability of our findings. Pregnant women were predominantly recruited to the BHIP study through Midwifery clinics. Findings from the Maternal Experiences Survey conducted November 2005 – May 2006 indicate that only 6.1% of pregnant women in Canada received midwife-led prenatal care.[147] Access to midwifery

care also varies greatly by province; 9.8% of women residing in British Columbia received midwife-led care whereas total prevalence in the Maritimes, Saskatchewan, and Yukon was only 0.3%. Furthermore, women who received prenatal care from a midwife were more likely to be educated, have aboriginal status, and drink alcohol during pregnancy. The majority of our participants were well educated. The high household income (>$75,000) and number of Caucasian women in our participant group may also limit generalizability to low-income populations or pregnant women of non-Caucasian ethnicity.

4.4 Contributions to clinical practice and future directions

Currently, pBMI is used by care practitioners as a screening tool for maternal obesity. Clinical practice guidelines in Canada state that obese pregnant women identified by pBMI should receive nutritional and weight gain counselling.[148] However, the use of pBMI as a screening tool presents major clinical concerns because this surrogate measure cannot distinguish body composition and often misrepresents true body fat distribution. Thus, it may not accurately indicate cardiometabolic risk in pregnancy. The lack of agreement on the optimal method to quantitatively assess body fat in pregnant women hinders the ability of policy makers to implement change to the guidelines. Our study has the potential to provide important information that could be applied to the decision-making process. In the clinical setting, BIA may be an ideal method because it is more easily adopted as it is faster and requires fewer technical skills by the operator than SFT measures.

Following the completion of recruitment to the BHIP study, the analysis of data beyond baseline can commence. We aim to compare the indirect quantitative measures of % body fat estimated from SFT and BIA to values measured directly by DXA at 6 months postpartum. This is important for determining whether indirect methods such as SFT and BIA are comparable to "gold-standard" measurements.

Future analyses by group allocation (i.e. intervention vs. control) will also be conducted to determine whether dietary and exercise intervention is causally associated with cardiometabolic biomarker profiles in pregnancy. Longitudinal analysis will determine whether diet and exercise intervention improves adverse cardiometabolic biomarker profiles in overweight and obese women across pregnancy and the postpartum.

Our study should provide detailed information about diet and PA level as these were objectively and quantitatively measured. A combination of multiple PA assessment methods is often recommended as no measure can capture all components of PA and combining methods can reduce limitations of each single method.[149] The BHIP study has employed this technique by collecting data from a self-reported exercise questionnaire, accelerometry, and pedometers. Daily step-count logs self-reported by participants in the intervention group were dually employed as a measure of PA and a motivation tool. Thus, good intervention compliance is anticipated which would increase the robustness of our findings.

Lastly, our study will assess the impact of maternal overweight and obesity and cardiometabolic status in pregnancy on infant health outcomes at birth and six months of age. The overall goal of future research is to determine whether new clinical practice

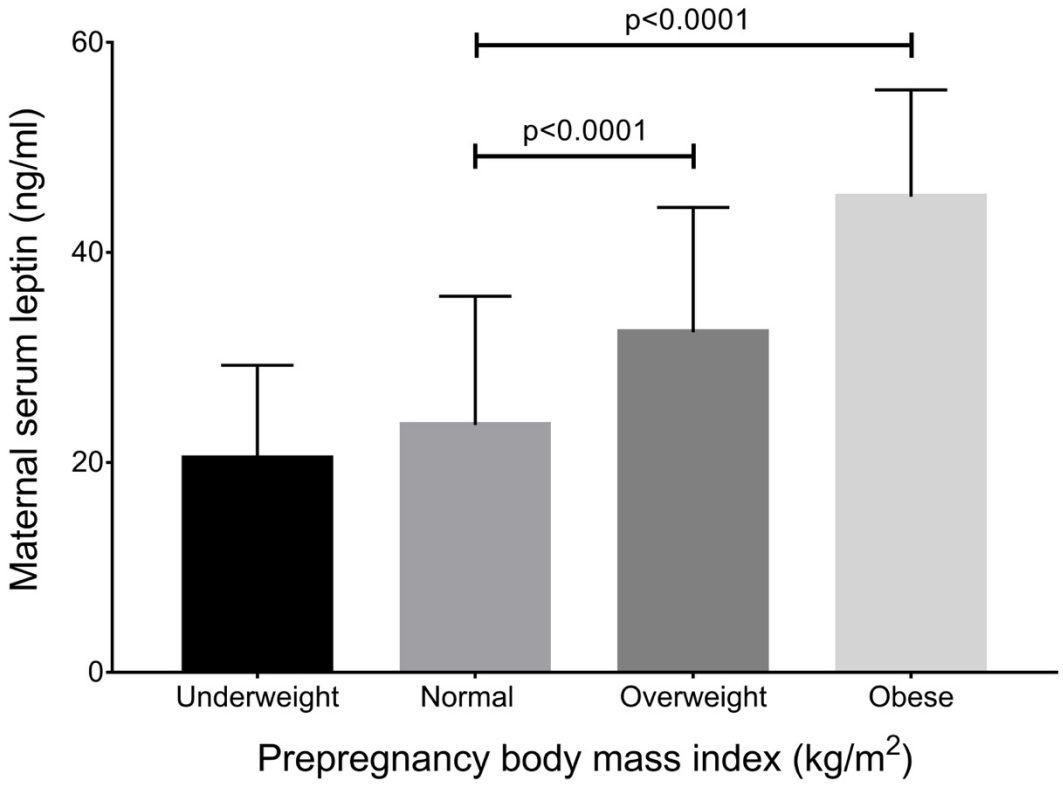
guidelines for screening of maternal adiposity and cardiometabolic dysfunction will improve infant health outcomes. The information gained from these future analyses will provide direction for future development of clinical practice guidelines on screening for maternal obesity and cardiometabolic risk in pregnancy.

CHAPTER 5

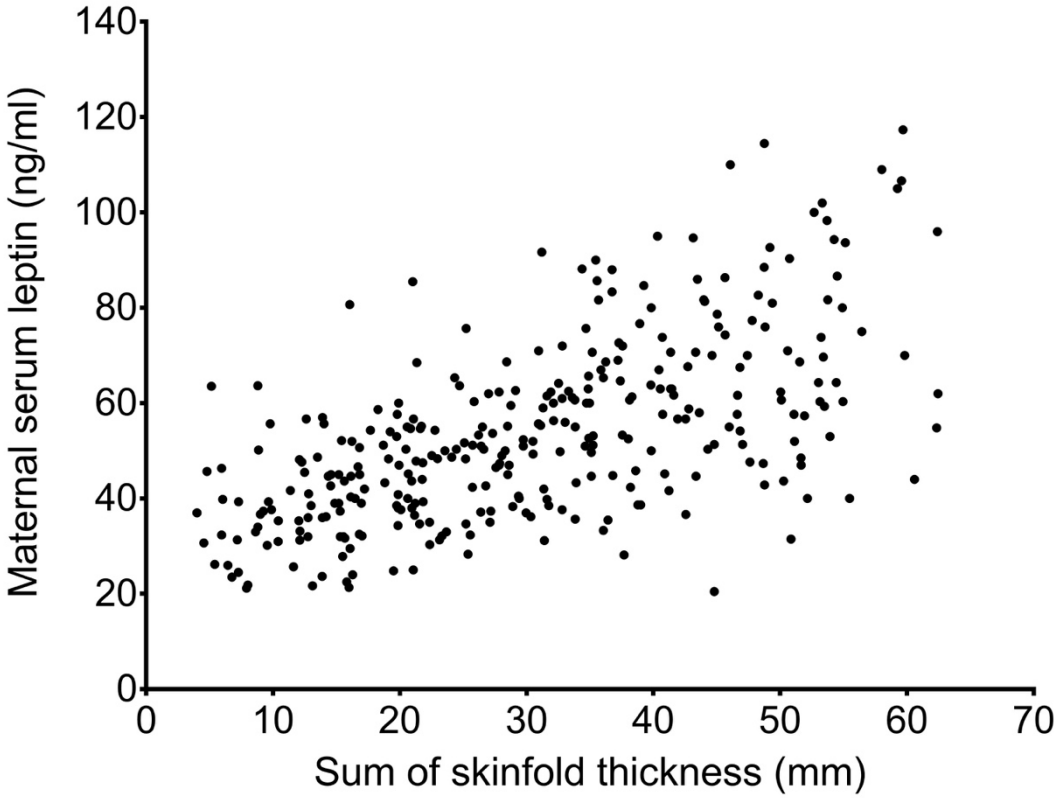
APPENDICES

CHAPTER 5- APPENDICES

5.1 Appendix 1 – Maternal adiposity and leptin in late pregnancy- Ancillary analysis of Family Atherosclerosis Monitoring In earLY Life (FAMILY) Study data



A comparison of leptin status by pre-pregnancy BMI using ANOVA. Maternal serum leptin concentration in the third trimester of pregnancy was higher in women with a prepregnancy BMI of overweight ($p<0.0001$) and obese ($p<0.0001$) compared to normal BMI



The association between maternal leptin concentration in the third trimester of pregnancy and 2-site SFT was measured using Pearson's correlation. There was a significantly positive association ($n=321$, $r= 0.6216$, $p<0.0001$).

5.2 Appendix 2 – Calculating PrimeScreen healthy eating score

Highlighted answers indicate healthful dietary behaviours (scored as 1). Other answers scored as 0 indicating less healthful behaviours.

Primescreen Nutrition Check

Study ID _____ (C####)

Date _____ (D-M-Y)

Questionnaire completed?
◯ Yes
◯ No

If questionnaire is not completed, please provide a reason: _____

The questions below are designed to help us understand your eating behaviors and food choices. There are no clear right or wrong answers. Please check the box that best describes your eating habits over the last month. How often do you eat...

1) Dark green leafy vegetables (spinach, romaine lettuce, mesclun mix, kale, turnip greens, bok choy, swiss chard):
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ Twice or more per day

2) Broccoli, brocoli rabe, cauliflower, cabbage, brussel sprouts:
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ Twice or more per day

3) Carrots:
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ Twice or more per day

4) Other vegetables (e.g. peas, corn, green beans, tomatoes, squash):
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ Twice or more per day

5) Citrus fruits (e.g. oranges, grapefruits):
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ Twice or more per day

6) Other fruits (e.g. fresh apples or pears, bananas, berries, grapes, melons):
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ Twice or more per day

7) Whole milk dairy foods (e.g. homogenized milk, hard cheese, butter, ice cream):
◯ Less than once per week
◯ Once per week
◯ 2 - 4 times per week
◯ Nearly daily or daily
◯ 2 - 3 times per day
◯ 4 - 6 times per day

8) Low-fat milk (e.g. skim, 1%, 2% milk):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ 2 - 3 times per day
- ◯ 4 - 6 times per day

9) Low fat Greek yogourt (0%, 2%):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ 2 - 3 times per day
- ◯ 4 - 6 times per day

10) Low fat regular yogourt:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ 2 - 3 times per day
- ◯ 4 - 6 times per day

11) Cottage cheese:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ 2 - 3 times per day
- ◯ 4 - 6 times per day

12) Fortified milk alternatives (e.g. soy, almond, rice milk)
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ 2 - 3 times per day
- ◯ 4 - 6 times per day

13) Whole eggs:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

14) Dried beans, split peas or lentils:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

15) Nuts and/or nut butter (e.g. peanut, almond, soy butters):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

16) Beef, pork or lamb:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

17) Processed meats (e.g. sausages, salami, bologna, hot dogs, bacon):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

18) Turkey or chicken:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

19) Fish/Seafood (not fried, but broiled, baked, poached, or canned):
- ◯ Less than once per month
- ◯ Once per month
- ◯ 2 - 3 times per month
- ◯ Weekly
- ◯ Twice or more per week

20) Refined grains (e.g. white bread, white rice):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

21) Whole grain breads and cereals (e.g. whole wheat, oatmeal, brown rice, barley):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

22) Baked products (e.g. muffins, doughnuts, cookies, cake, pastries):
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

23) Sugar-sweetened beverages (e.g. regular soda, fruit drinks, Nestea, Gatorade)
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

24) Deep fried foods:
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

25) How often do you add salt to food at the table?
- ◯ Less than once per week
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ Nearly daily or daily
- ◯ Twice or more per day

26) Have you searched for information about health in pregnancy from any of the following:
- ☐ Public Health prenatal classes
- ☐ Other prenatal course/workshop
- ☐ Canadian Government website
- ☐ Local Public Health website
- ☐ Other health care organization's website
- ☐ Smart phone application (app)
- ☐ Other
- ☐ None

(Check all that apply)

If you checked any item in question 26, please describe below:

Public Health prenatal classes _____ (Please describe)

Other prenatal course/workshop _____ (Please describe)

Canadian Government website _____ (Please describe)

Local Public Health website _____ (Please describe)

Other health care organization's website _____ (Please describe)

Smart phone application (app) _____ (Please describe)

Other _____ (Please describe)

5.3 Appendix 3 – Multiple regression results for all outcomes and independent variables

	Outcomes									
	Glucose (mM)		**Insulin † (μIU/L)**		**Triglycerides † (mg/dL)**		**Leptin † (ng/mL)**		**Adiponectin † (μg/mL)**	
Independent variables	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Percent body fat (from SFT)	**1.11 (0.47, 1.74)**	**<0.01**	**1.93 (1.02, 2.83)**	**<0.001**	**0.88 (0.28, 1.47)**	**<0.01**	**3.33 (2.39, 4.26)**	**<0.001**	**-0.81 (-1.38, -0.25)**	**<0.01**
Age (years)	0.00 (-0.02, 0.03)	0.71	**-0.04 (-0.07, -0.01)**	**<0.05**	0.01 (-0.01, 0.03)	0.50	-0.03 (-0.06, 0.01)	0.11	0.01 (-0.01, 0.03)	0.20
Ethnicity (non-Caucasian)	-0.26 (-0.53, 0.02)	0.06	0.24 (-0.15, 0.63)	0.23	**0.27 (0.01, 0.52)**	**<0.05**	-0.30 (-0.71, 0.10)	0.14	-0.07 (-0.31, 0.16)	0.53
Parity	–	–	–	–	–	–	–	–	–	–
Primiparous	0.00 (-0.18, 0.19)	0.99	-0.02 (-0.28, 0.24)	0.89	0.05 (-0.12, 0.22)	0.55	-0.09 (-0.36, 0.18)	0.52	-0.15 (-0.32, 0.03)	0.10
Multiparous	0.06 (-0.16, 0.28)	0.57	0.11 (-0.20, 0.42)	0.47	0.05 (-0.16, 0.25)	0.65	-0.00 (-0.32, 0.32)	0.99	**-0.29 (-0.47, -0.11)**	**<0.01**
Energy expenditure (kcal/d) †	0.52 (-0.21, 1.25)	0.16	**1.78 (0.74, 2.82)**	**<0.01**	0.19 (-0.49, 0.88)	0.57	**1.48 (0.41, 2.55)**	**<0.01**	-0.16 (-0.90, 0.57)	0.66
MVPA (minutes/d) ‡	-0.03 (-0.07, 0.01)	0.13	**-0.06 (-0.12, -0.00)**	**<0.05**	-0.02 (-0.06, 0.02)	0.32	-0.03 (-0.09, 0.03)	0.26	0.02 (-0.02, 0.06)	0.35
Total energy intake (kcal/d)	-0.00 (-0.00, 0.00)	0.81	-0.00 (-0.00, 0.00)	0.19	0.00 (-0.00, 0.00)	0.23	-0.00 (-0.00, 0.00)	0.63	-0.00 (-0.00, 0.00)	0.60
P:S ratio ‡	**0.90 (0.33, 1.47)**	**<0.01**	-0.51 (-1.32, 0.31)	0.22	0.11 (-0.42, 0.65)	0.68	**-0.94 (-1.78, -0.10)**	**<0.05**	-0.22 (-0.71, 0.27)	0.37

†Values log transformed prior to regression analysis

‡Values square root transformed prior to regression analysis

Independent variables	Outcomes									
	Glucose (mM)		**Insulin † (μIU/L)**		**Triglycerides † (mg/dL)**		**Leptin † (ng/mL)**		**Adiponectin † (μg/mL)**	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Percent body fat (from BIA)	**0.03 (0.01, 0.05)**	**<0.01**	**0.03 (0.00, 0.06)**	**<0.05**	**0.02 (0.00, 0.04)**	**<0.05**	**0.08 (0.04, 0.11)**	**<0.001**	-0.02 (-0.03, -0.00)	0.08
Age (years)	0.00 (-0.03, 0.02)	0.72	**-0.05 (-0.08, -0.01)**	**<0.02**	0.00 (-0.02, 0.02)	0.87	**-0.05 (-0.08, 0.01)**	**<0.05**	0.02 (-0.00, 0.04)	0.13
Ethnicity (non-Caucasian)	-0.24 (-0.51, 0.04)	0.09	0.29 (-0.12, 0.71)	0.17	**0.29 (0.02, 0.55)**	**<0.05**	-0.23 (-0.68, 0.23)	0.33	-0.11 (-0.35, 0.13)	0.35
Parity	–	–	–	–	–	–	–	–	–	–
Primiparous	-0.03 (-0.22, 0.15)	0.71	-0.06 (-0.34, 0.23)	0.69	0.03 (-0.15, 0.20)	0.75	-0.17 (-0.48, 0.13)	0.27	-0.11 (-0.29, 0.07)	0.24
Multiparous	0.07 (-0.15, 0.29)	0.52	0.10 (-0.23, 0.44)	0.53	0.05 (-0.16, 0.26)	0.64	-0.00 (-0.36, 0.36)	0.99	**-0.29 (-0.48, -0.10)**	**<0.01**
Energy expenditure (kcal/d) †	-0.01 (-0.95, 0.94)	0.99	**1.72 (0.28, 3.15)**	**<0.05**	-0.06 (-0.95, 0.84)	0.90	0.72 (0.83, 2.28)	0.36	-0.15 (-1.08, 0.77)	0.74
MVPA (minutes/d) ‡	-0.02 (-0.07, 0.02)	0.32	**-0.08 (-0.15, -0.01)**	**<0.05**	-0.02 (-0.06, 0.02)	0.38	-0.04 (-0.11, 0.03)	0.29	0.03 (-0.02, 0.07)	0.23
Total energy intake (kcal/d)	-0.00 (-0.00, 0.00)	0.68	-0.00 (-0.00, 0.00)	0.21	0.00 (-0.00, 0.00)	0.28	-0.00 (-0.00, 0.00)	0.56	-0.00 (-0.00, 0.00)	0.73
P:S ratio ‡	**1.00 (0.41, 1.56)**	**<0.01**	-0.37 (-1.24, 0.50)	0.40	0.18 (-0.37, 0.72)	0.52	-0.70 (-1.64, -0.25)	0.15	-0.27 (-0.78, 0.24)	0.29

†Values log transformed prior to regression analysis

‡Values square root transformed prior to regression analysis

5.4 Appendix 4 – Comparison of body fat content measurement methods in pregnant women by Nicole Azizian – BSc (Biology); fourth year thesis March 2014

SFT and BIA measurement time (minutes) from pregnant women (n=14) enrolled in the BHIP study at 26 – 28 weeks gestation. Data presented as mean ± S.D when excluding set-up time

	Measurement time (minutes)	
	SFT	**BIA**
Excluding set-up time	5.15 ± 0.80	0.21 ± 0.15
Including set-up time	5.15	0.51

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