# MATERNAL ADIPOSITY CHANGES DURING PREGNANCY

# MATERNAL BODY ADIPOSITY CHANGES DURING PREGNANCY AND ASSOCIATION WITH CARDIOMETABOLIC STATUS AND ADVERSE OUTCOMES IN A RANDOMIZED NUTRITION+EXERCISE INTERVENTION TRIAL

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## LAY ABSTRACT

Pregnancy is associated with a natural gain in body fat, but it can reach excessive amounts. Excess body fat is of clinical consequence as it is associated with poor cardiovascular health and abnormal pregnancy outcomes. Improving diet and physical activity habits may reduce excess weight gain, but little is known about how it influences fat gained during pregnancy. In our study body fat gain during pregnancy was similar between the lifestyle intervention and control groups. However, entering pregnancy with greater BMI was associated with less fat gain during pregnancy. Changes in body fat influenced cardiovascular blood markers, but results differed between body fat assessment tools. We also found that methods to measure body fat produce different results at different stages of pregnancy. Our findings provide insight on the factors that influence fat gain during pregnancy and highlight the need for better tools to measure body fat accurately in pregnancy.

ABSTRACT

**Rationale & Background:** Gaining excessive adiposity in pregnancy is associated with altered cardiometabolic profile and adverse pregnancy outcomes. Lifestyle interventions may reduce excess weight gain, but the effect on fat gain is unclear. Our study explored this question by 1) comparing measures of body fat (BF) by bioelectrical impedance analysis (BIA) and 4-site skinfold thickness (SFT); 2) assessing the impact of a nutrition+exercise intervention on adiposity changes; 3) elucidating associations between adiposity changes and cardiometabolic biomarkers and adverse pregnancy outcomes.

**Study Design:** Participants randomized to receive a high dairy protein diet and exercise program (intervention) or standard care (control) in the Be Healthy in Pregnancy RCT (NCT 01689961) had adiposity measured at 12-17, 26-28, and 36-38 weeks gestation by BIA (%BF) and SFT (sum and %BF), and at 6 months postpartum also by DXA. Fasted blood samples collected at 12-17 and 36-38 weeks gestation were analyzed for glucose, lipid profile, insulin, leptin, adiponectin, and CRP. Pregnancy outcomes were abstracted from medical charts.

**Results:** In 181 participants, BIA %BF and SFT %BF had good agreement in early pregnancy and postpartum, but low agreement in late pregnancy. Adiposity changes across pregnancy were similar between study arms but were greater in normal weight compared to overweight women. Insulin and leptin were negatively associated with change in SFT (sum and %BF). Triglycerides were negatively associated with change in

BIA %BF, while HDL was positively associated. Neither caesarean section nor operative vaginal delivery were associated with adiposity change.

**Conclusion:** Adiposity measured by sum of SFT and BIA %BF increased across pregnancy but was not influenced by the diet+exercise intervention. Associations of adiposity change with cardiometabolic biomarkers varied between measurement tools. The lack of adiposity measurement tools appropriate across pregnancy and in clinical settings presents a concern for assessing clinical responses to adiposity change across pregnancy.

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LIST OF ABBREVIATIONS

APrON – Alberta Pregnancy Outcomes and Nutrition

BD – body density

BHIP – Be Health in Pregnancy

BIA – bioelectrical impedance analysis

BMI – body mass index

CRP – c-reactive protein

DBP – diastolic blood pressure

DXA – dual energy x-ray absorptiometry

EDTA – ethylenediaminetetraacetic acid

ELISA – enzyme linked immunosorbent assay

FAMILY – Family Atherosclerosis Monitoring In early Life

FFM – fat free mass

FFQ – food frequency questionnaire

FM – fat mass

GDM – gestational diabetes mellitus

GEE – generalized estimating equations

GWG – gestational weight gain

HDL – high density lipoprotein

IOM – Institute of Medicine

LDL – low density lipoprotein

Life-MOM – Lifestyle Interventions For Expecting MOMs

pBMI – pre-pregnancy body mass index

PUFA:SFA – polyunsaturated fatty acid to saturated fatty acid ratio

SBP – systolic blood pressure

SFT – sum of skinfold thickness

TBW – total body water

WHO – World Health Organization

%BF – percent body fat

# CHAPTER 1

# INTRODUCTION

# CHAPTER 1 – INTRODUCTION

## 1.1 CLINICAL PROBLEM: EXCESSIVE GESTATIONAL WEIGHT GAIN (GWG)

### 1.1.1 PREVALENCE OF EXCESSIVE GWG AMONG CANADIANS

Excessive gestational weight gain (GWG) during pregnancy is prevalent among more than 60% of Canadian women who exceed the Institute of Medicine (IOM) GWG recommendations (Table 1) during their pregnancy, with even higher prevalence among women entering pregnancy overweight or obese (1–4). A prospective cohort study conducted in 2012 called Alberta Pregnancy Outcomes and Nutrition (APrON) reported more than 75% of overweight or obese women exceeded GWG guidelines during their pregnancy (5). A higher pre-pregnancy body mass index (pBMI) was strongly associated with exceeding gestational weight gain guidelines (5), which is of particular concern given the increasing prevalence of pregravid obesity. A prospective birth cohort study called Family Atherosclerosis Monitoring in Early Life (FAMILY) reported more than 50% of Canadian women are entering pregnancy overweight or obese (6).

**Table 1** Institute of Medicine gestational weight gain guidelines.

| **Pre-pregnancy BMI** | **BMI (kg/m^2) (WHO)** | **Total Weight Gain Range** | **Rates of Weight Gain 2nd and 3rd trimester* (mean range lb/week)** |
|---|---|---|---|
| Underweight | <18.5 | 28 – 40 | 1 (1 – 1.3) |
| Normal Weight | 18.5 – 24.9 | 25 – 35 | 1 (0.8 – 1) |
| Overweight | 25.0 – 29.9 | 15 – 25 | 0.6 (0.5 – 0.7) |
| Obese (all classes) | ≥30.0 | 11 - 20 | 0.5 (0.4 – 0.6) |

*Calculations assume a 1.1 – 4.4 lbs (0.5 – 2.0 kg) weight gain in the first trimester.

### 1.1.2 ADVERSE HEALTH OUTCOMES ASSOCIATED WITH EXCESSIVE GWG

Excessive GWG and pregravid obesity both present major clinical concerns due to their association with greater risk of adverse outcomes for the mother during pregnancy,

as well as adverse outcomes later in life for both the mother and her child (7–9). Pregravid obesity and excessive gestational weight gain can increase a mother's risk for developing gestational diabetes (GDM) and hypertensive disorders such as preeclampsia (8,10–12). A recent systematic review and meta-analysis of 13 studies (N = 156170) found that excessive gestational weight gain was associated with greater risk of hypertensive disorders, such as preeclampsia, independent of pBMI (8). Regarding GDM, a systematic review of 8 studies (N = 13748) reported a 40% greater risk of GDM in women who gain excessive weight during pregnancy compared to those with GWG within the guidelines. Excessive GWG and pregravid obesity are also associated with a higher risk of perinatal outcomes, such as caesarean delivery (13,14). These adverse maternal and perinatal outcomes can have lasting health impacts post-partum, increasing the risk of stroke, obesity, and Type II diabetes later in life (15,16). For the infant, risk of macrosomia rises with maternal obesity and excessive GWG (17). Importantly, excessive GWG is acknowledged as the strongest predictor for childhood obesity (18,19).

## 1.2 Clinical effectiveness of lifestyle interventions on excessive GWG

### 1.2.1 Effect of lifestyle interventions on GWG and Adverse Outcomes

Given the benefit lifestyle interventions have on mediating weight gain and reducing risk of adverse outcomes in non-pregnant populations (20–24), several studies have explored the impact of lifestyle interventions during pregnancy on reducing excessive GWG and reducing risk of adverse outcomes (25–29). Current evidence suggests that lifestyle interventions can reduce GWG, however variation between interventions results in inconsistent evidence. In a randomized trial of 962 women, light to moderate aerobic

and resistance exercise resulted in a significant reduction in GWG compared to the control group (30). However, a large scale randomized trial (N = 2212) evaluating the effect of comprehensive diet, exercise, and behaviour advice on GWG found no significant treatment effect on GWG (31). Further, while lifestyle interventions may reduce the amount of weight gained, many women still remain above guidelines. A review of 49 randomized controlled trials (N= 11444),showed that diet and exercise interventions reduced the risk of excess GWG during pregnancy by 20% when compared to standard care in the 24 studies reporting GWG (29). Despite this significant reduction in risk, however, 38% of women in diet and exercise interventions still exceeded GWG guidelines. Similarly, in a prospective meta-analysis of a consortium of 7 randomized controlled trials with various lifestyle interventions, risk of weekly excessive GWG was significantly reduced. However, more than 60% of women receiving the intervention remained above recommended GWG (25).

Whether lifestyle interventions during pregnancy reduces the risk of adverse outcomes such as GDM, preeclampsia, caesarean delivery, and macrosomia, remains controversial. A randomized controlled trial in obese women found dietary counselling and physical activity reduced GWG, however risk for GDM, preeclampsia, and caesarean section were similar between study arms (32). Similarly, in a systematic review and meta analysis evaluating the impact of GWG on risk of adverse outcomes among women following lifestyle interventions (N – 1732), the authors found a reduction in GWG following a diet and exercise intervention but with no significant effect on risk for GDM, preeclampsia, or pregnancy-induced hypertension. However, another systematic review

and meta-analysis of 36 randomized controlled trials (N = 6543) found that lifestyle interventions were associated with a reduced risk of preeclampsia (33). Taken together, while lifestyle interventions may be effective at reducing excessive GWG, it is inconclusive whether such interventions reduce the risk of adverse outcomes associated with GWG.

### 1.2.2 Body composition as a marker of the effectiveness of lifestyle interventions

In evaluating the effectiveness of lifestyle interventions, it is important to consider not only the amount of weight gained but the composition as well. Adiposity, in particular, is important to evaluate due to its relationship to cardiometabolic profile, maternal pregravid adiposity, and adverse outcomes. Maternal cardiometabolic adaptations across pregnancy contribute to changes in fat mass (FM) and fat-free mass (FFM) (34,35). These changes reflect the changing energy demands across pregnancy by ensuring appropriate energy utilization and storage (36,37). However, as women gain more weight during their pregnancy, there are proportional increases in body fat, regardless of whether they gain an appropriate or excessive amount of weight (38). As well, a study found that overweight and obese women who gained an excessive amount of weight had greater FM than women who gained an appropriate amount of weight, but there were no significant differences in lean mass (39). These findings suggest that excessive GWG is predominately FM. Thus, evaluating the effect of lifestyle interventions on the amount of fat gained is of interest as it is unclear whether reductions in GWG by lifestyle interventions are reflected in changes in adiposity.

A challenge in evaluating the effect lifestyle interventions on changes in adiposity is that available measures of body adiposity have limitations when used in pregnancy. Pregnancy is a dynamic state, with significant fluid shifts across pregnancy (40). This is a concern for two- compartment measurement tools, such as bioelectrical impedance analysis (BIA), as these tools are not validated in pregnancy and assume a constant hydration (40,41). Hydration changes also influences compressibility of tissues, impacting tools such as ultrasound and sum of skinfolds (SFT) (40). Some measurement tools also offer an estimate of total maternal body fat by measuring particular body sites and fat depots (40,41). Given that maternal fat deposition in pregnancy is not uniform across the body, the estimate of total body adiposity is biased based on where the measurement was taken and what fat depots were measured (42–44). Further, these measurement tools cannot separate fetal and maternal contributions to total body fat (40,41). While imaging tools, such as magnetic resonance imaging (MRI) and dual energy x-ray absorptiometry (DXA), can address many of the limitations present in most two-compartment models of body composition, these tools may not be appropriate in clinical trials due to the financial cost or safety (40,41). Dual-energy x-ray absorptiometry in particular cannot be used due to risk of exposing the fetus to radiation (40,41). The varying limitations between measurement tools when used in pregnancy give rise to speculation that absolute measures of adiposity will differ between measurement tools. As part of evaluating the effect of lifestyle interventions on changes in maternal adiposity, it is then important to consider how adiposity was measured and how the tool's limitations may impact findings.

### 1.3 Impact of adiposity in pregnancy

Pregravid obesity is associated with an altered cardiometabolic profile, which may impact maternal health, pregnancy outcomes, and infant health. Women who are overweight or obese enter pregnancy with a more atherogenic profile when compared to normal weight women (45). Further, changes in lipid metabolism across pregnancy differ between pBMI categories, with a smaller increase in total cholesterol, low density lipoprotein (LDL), and triglycerides among overweight and obese women when compared to normal weight women (46–48). In regards to glucose metabolism, in one study in early pregnancy, the expected drop in maternal glucose lessened with increasing pBMI, with obese women showing increases in circulating glucose concentrations (49). As well, insulin resistance was further exacerbated in heavier women, resulting in hyperinsulinemia (36,48). Adipokines leptin and adiponectin are both greatly influenced by pregravid obesity and maternal adiposity. Leptin concentrations are significantly greater in obese women when compared to lean women, while adiponectin concentrations are significantly lower (50,51).

Greater adiposity during pregnancy is also associated with adverse health outcomes for the mother. Having greater body fat during pregnancy is associated with adverse outcomes such as elevated inflammation, increased risk of gestational hypertension, and increased insulin resistance (52–54). Adiposity can also influence birth outcomes as women with greater pBMI are at a greater risk for pregnancy complications and caesarean delivery (55).

While evidence does suggest that greater adiposity is associated with an altered cardiometabolic profile and increased risk of adverse outcomes, there is limited evidence regarding how fat accumulation during pregnancy influences these clinical outcomes. In a small observational study (n = 16) of pregnant women with normal and abnormal glucose metabolism, the authors found that a greater change in body fat from preconception to early pregnancy was associated with a decrease in relative insulin sensitivity (56). However, associations between fat accumulation and lipid profile, adipokines, and inflammatory biomarkers are not well understood. Further, the impact of change in maternal adiposity on risk of adverse outcomes is unclear. Therefore, it was of interest in my research to evaluate how changes in maternal adiposity were related to cardiometabolic biomarker concentrations and risk of adverse outcomes in pregnancy.

### 1.4 Gaps in knowledge identified

Systematic reviews of lifestyle intervention trials in pregnancy have demonstrated that nutrition and/or exercise or behavioural interventions are effective in reducing GWG in pregnant women; however, there is wide heterogeneity across trials and GWG often remains above recommendations (25,29,57). Further, there is a growing body of literature that suggests the reductions in GWG achieved may or may not reduce the risk of adverse maternal and fetal outcomes (32,33,58). In a prospective meta-analysis of the Lifestyle Interventions For Expecting Moms (Life-MOM; a consortium of seven independently run randomized controlled trials across the United States), the authors concluded there is a need to explore the impact of lifestyle interventions on body composition (fat and lean mass) before conclusions can be made on the clinical significance of lifestyle

interventions on GWG. To date, the factors influencing body fat accumulation during pregnancy and the optimal measurement tool have not been well explored. As well, cardiometabolic adaptations in pregnancy are both influenced by and result in changes in body composition. Pre-gravid obesity and adiposity in pregnancy are associated with abnormal cardiometabolic status and poor health outcomes, however the impact of changes in maternal adiposity on cardiometabolic profile and risk of adverse events is unclear (45,49,52–54). The key focus of my research was to assess the impact of a nutrition+exercise intervention on the composition of weight gain measured as FM throughout pregnancy and determine the association with biomarkers of maternal cardiometabolic status and maternal health outcomes.

### 1.5 Objectives and hypotheses

In my approach to address the proposed research question, I conducted a methods analysis where my objective was:

1. To conduct a comparative analysis between bioelectrical impedance analysis (BIA) and sum of skinfold thickness (SFT) as measures of percent body fat (%BF) as an essential first step in providing guidance as to which measure should be used to assess further associations.

Following this, to address the proposed research question, I conducted an intervention analysis, as well as an assessment of clinical outcomes. The objectives for this part of the analysis were:

1. To evaluate the impact of the intervention on maternal body composition by comparing changes in body fat to those in the control group. The aim is to examine the change in body fat across each of the two trimesters of pregnancy accounting for adherence to the intervention, as well as evaluate if there were any lasting changes at six months post partum
2. To elucidate the relationship between changes in maternal adiposity and cardiometabolic biomarkers in early and late pregnancy.
3. To elucidate the relationship between changes in maternal adiposity and immediate adverse maternal health outcomes.

I hypothesize that women following the nutrition+exercise intervention will have a smaller increase in FM during pregnancy, more optimal cardiometabolic profile, and fewer adverse health outcomes compared to those not participating in this lifestyle intervention.

# CHAPTER 2

# STUDY DESIGN AND METHODS

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## A: BE HEALTHY IN PREGNANCY STUDY DESIGN

This study addresses a secondary objective of the Be Healthy in Pregnancy (BHIP) study, a two-arm, two-site CIHR-funded prospective randomized controlled trial (registered at clinicaltrials.gov as NCT01689961). The primary objective of BHIP is to determine whether a structured nutrition and exercise program will increase the likelihood of women attaining gestational weight gain within the Institute of Medicine guidelines when compared to standard prenatal care. Details of the study design have been published (59). Briefly, from January 2013 to March 2018, healthy pregnant women between 12-17 weeks gestation (n = 241) were recruited and followed throughout pregnancy and to 6 months postpartum. Recruitment of participants in the Hamilton, London, and Burlington area was facilitated by healthcare professionals, as well as poster advertisements in both healthcare and community settings. Eligibility to participate was evaluated through a telephone interview following the criteria presented in Table 2. Eligible participants interested in the study attended an initial study visit between 12-17 weeks gestation at which baseline measures were collected, health questionnaires were conducted, and initial consent was obtained. At a second study visit, consented participants were randomized (by telephone/electronic randomization) to either the intervention arm or control arm in a 1:1 ratio, stratified for study site and pBMI. Women in the control group (n = 119) were provided standard prenatal care along with counseling that is in line with the Health Canada Guide on Prenatal Nutrition (60). Women in the intervention group (n = 118) received the same treatment as control, with the addition of a higher protein (25% of

energy intake) nutrition plan individualized to participant estimated energy requirements and focused on dairy sources of protein that were provided bi-weekly, along with a monitored walking program with a goal of 10,000 steps per day. Participants in the intervention groups attended weekly or biweekly sessions which involved a personalized counselling session with study nutritionist, a walk with study staff to reach step goal, and retrieval of low fat dairy foods (59). Four women were randomized but were immediately withdrew from the study thus no outcome data were available for these women, so they could not be included in the per protocol analysis.

**Table 2** Inclusion and exclusion criteria for the Be Healthy in Pregnancy Study.

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| • Aged > 18 years<br>• Singleton pregnancy<br>• Able to be randomized to group allocation by 17 weeks and six days of gestation<br>• Pre-pregnancy body mass index < 40 $kg/m^2$<br>• Planning to deliver at a Hamilton, Burlington, or London regional hospital or by home birth and willing to attend research visit at either study site<br>• Approval of primary care provider to participate in exercise<br>• Able to provide signed informed consent | • Not conversant in English<br>• Pre-existing diabetes<br>• Severe chronic gastrointestinal, heart, kidney, liver, of pancreatic diseases or conditions<br>• Refusal to consume dairy foods<br>• Known contraindications to exercise as recommended by the Canadian clinical practice guidelines for pregnancy<br>• Currently smoking and will not discontinue during the pregnancy<br>• Depression score greater than 12 on the validated Edinburgh Depression scale |

## B: Comparative analysis of adiposity measures in pregnancy

### B.2.1 Anthropometric and body composition measurements

Height and weight were measured at enrollment before randomization. Height was measured using a wall-mounted stadiometer (Ellard Instrumentation, Monroe WA).

Weight was measured using a Tanita® BF-350 Body Composition Analyser (Arlington Heights, IL). Pre-gravid weight was estimated as weight measured at study entry minus self-reported gestational weight gain. Pre-pregnancy body mass index was calculated using pre-gravid weight and height measured at enrollment (59).

Maternal adiposity was assessed by leg-to-leg bioelectrical impedance analysis (BIA) and sum of 4-site (triceps, biceps, sub-scapular, suprailiac crest) skinfold thickness (SFT) at 12-17, 26-28, 36-38 weeks. At 6 months post-partum, BIA and SFT were also measured along with whole-body dual energy x-ray absorptiometry (DXA) (59). Measures of body fat by 4-site SFT and leg-to-leg BIA were used to evaluate whole body adiposity using a two-compartment model for body composition that separated body mass into FM or FFM. As a two-compartment model, FM measures are estimated based on body hydration and thus measurements are influenced by changes in hydration. Dual energy x-ray absorptiometry evaluated adiposity using a three-compartment model for body composition that separated body mass into FM, mineral mass, and FFM. For DXA, a QDR®4500 series Hologic Inc. Discovery™ dual energy x-ray absorptiometry machine was used with Adult whole body software version 12.3.1 (Waltham, MA) at the McMaster University study site, and the General Electric-Luna iDXA was used with Amex Medical encore software version 14.1 at the Western University study site (59). Dual energy x-ray absorptiometry emits two low dose x-rays at different energies and measures a ratio of the natural logarithms of the ratio of unattenuated and attenuated x-rays, which is unique to different tissues and can be used to estimate tissue amounts (61). Quality control tests were conducted daily using an artificial $L_{1-4}$ lumbar spine made of

hydroxyapatite in epoxyresin. Measurements had to be within ±1.0% of known mineral content values to pass quality control tests. Further, a weekly calibration test was conducted using a step phantom composed of materials similar in density to soft and lean tissue. A weekly uniformity test was also conducted to evaluate the attenuation of the X-rays (59). For BIA, a Tanita® BF-350 Body Composition Analyser was used (Arlington Heights, IL). This scale measures adiposity by sending a single frequency electrical current up through the feet and legs up to the lower abdomen and assessing the resistance to current flow using assumptions on total body water (TBW), body shape, and fat fraction (40,62). The greater the water content of the specific tissue, the more conductive that tissue is and the lower its resistance (62). Due to the current flow moving through the body, BIA measures both subcutaneous and visceral fat depots. Proprietary equations from Tanita® were used to directly calculate percent body fat from resistance, adjusting for gender, age, height, and weight to improve accuracy. Equations used for BIA were not specific to pregnancy and were validated in non-pregnant populations against DXA (Arlington Height, IL). Skinfolds were conducted using a Harpenden skinfold caliper at the triceps, biceps, sub-scapular, and suprailiac crest skinfold sites. Triplicate measures were taken to improve precision. These measures were averaged for each site and then added to produce a sum of skinfolds. Given how skinfolds are measured, SFT measures only subcutaneous fat depots. Sum of skinfolds was also converted to percent body fat by first converting sum of skinfolds to body density (BD) (63). This specific equation has not been validated in a pregnant population. These density values were then used to calculate %BF using equations validated for specific gestational ages against a four

compartment model (64). A conversion equation specific to non-pregnant women was used for values measured at 6 months postpartum (64). The conversion to equations used are presented below:

$$BD = 1.1581 - 0.0720(\log[sum\ of\ skinfolds])$$

$$\%BF\ at\ 12\ weeks = \frac{4.97}{\mathrm{BD} - 4.523}$$

$$\%BF\ at\ 24\ weeks = \frac{5.043}{\mathrm{BD} - 4.604}$$

$$\%BF\ at\ 36\ weeks = \frac{5.163}{\mathrm{BD} - 473.7}$$

$$\%BF\ not\ specific\ to\ pregnancy = \frac{4.95}{\mathrm{BD} - 4.50}$$

B.2.2 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 26.0 (IBM Corp, 2016, Armonk, NY, USA). Descriptive statistics were presented as mean and standard deviation for normally distributed variables or as median and quartiles (Q1 and Q3) for non-normally distributed variables. Adiposity measures across pregnancy were examined using Spearman's Coefficient. To evaluate differences between time-points, repeated measures one-way ANOVA and Friedman's Test for normal and non-normal data, respectively. Statistical significance for ANOVA and Kruskal-Wallis's test was defined as a two-sided p-value of $<0.05$.

For the comparative analysis, the agreement between adiposity measures and the ability to discriminate between normal weight and overweight/obese was evaluated. To evaluate the agreement between adiposity measures at 12-17, 26-28, and 36-38 weeks

gestation, as well as 6 months postpartum, a Bland-Altman plot was produced using GraphPad Prism 7.03 (GraphPad Software, Inc., La Jolla, CA, USA). Pearson correlation and Lin's concordance correlation coefficient were also calculated (65). McNemar's test was used to compare the ability to discriminate between normal weight and overweight/obese between adiposity measures at 12-17, 26-28, 36-38 weeks gestation, as well as 6 months postpartum (66). McNemar's test was also used to compare discrimination power between adiposity tools and pre-pregnancy body mass index. The C-statistic was used to evaluate the concordance between pBMI classification and adiposity measures (67). For %BF estimated by BIA, SFT, and DXA, weight categories defined by %BF were used. Normal weight was defined as less than 31% body fat while overweight/obese was defined as greater than or equal to 31% body fat (68). These values were derived from a non-pregnant population as no values currently exist for a pregnant population. For pre-pregnancy body mass index, weight categories defined by body mass index were used where normal weight was defined as $< 25$ kg/m$^2$ and overweight/obese was defined as $\geq 25$ kg/m$^2$. Statistical significance of Pearson correlation, Lin's concordance correlation coefficient, and McNemar's test was defined as a two-sided p-value of $<0.05$.

### C: Intervention study: clinical outcome measures

#### C.2.1 Assessment of maternal cardiometabolic status

To assess cardiometabolic profile, blood samples were collected at two time points: first between 12 and 17 weeks gestation and again between 36 and 38 weeks gestation following a 12 hour fast. A volume of 19.5 mL was collected and aliquoted into 4 tubes:

sodium fluoride/ $Na_2$ ethylenediaminetetraacetic acid (EDTA) (2mL); PAXgene® Blood RNA tube (PreAnalytix) (2.5mL); SST™ Serum Separation Tubes with gel (BD Vacutainer®) (5mL) and silicone coated serum tube (BD Vacutainer®) (10mL). Samples were left to clot at room temperature for 30 minutes except for the PAXgene® aliquot, which was left at room temperature for a minimum of 2 hours. The silicone coated serum tube, and sodium fluoride/$Na_2$ EDTA tube were centrifuged at 3000rpm for 10 mints at 4°C. The SST™ serum separation tube was centrifuged for 15 minutes at 4°C. Samples were then aliquoted into polypropylene microcentrifuge tubes and stored at -20°C for a minimum of 24 hours before being transferred to an -80°C freezer (59).

Samples were analyzed for glucose, lipid profile, insulin, leptin, adiponectin, and C-reactive protein. Fasting plasma glucose and lipid profile biomarkers (triglycerides, total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL)) were measured using assays completed by the Hamilton Health Sciences Regional Laboratory Medicine Program. Insulin, leptin, adiponectin, and C-reactive protein (CRP) were analyzed using Luminex® human premixed multi-analyte enzyme-linked immunosorbent assay (ELISA) in the Atkinson Lab by trained staff, not including myself (59). Reference values for cardiometabolic biomarkers can be found in Table 3 for early pregnancy and Table 4 for late pregnancy reference ranges. The specifics regarding the assays used to estimate the concentrations of the outlined biomarkers are provided in Appendix 1.

**Table 3** Reference ranges for cardiometabolic biomarkers in early and late pregnancy for a healthy pregnant woman.

| Analyte | Reference Range in Early Pregnancy | Citation |
|---|---|---|
| Glucose (mmol/L)[1] | 4.1 – 5.5 | Laboratory (69) |
| Insulin (pmol/L) | 34.31 – 70.97 | Literature (70) |
| Total Cholesterol (mmol/L) | 7.8 – 11.7 | Laboratory (71) |
| HDL (mmol/L) | 2.2 – 4.3 | Laboratory (71) |
| LDL (mmol/L) | 3.3 – 8.5 | Laboratory (71) |
| Triglycerides (mmol/L) | 2.2 – 8.8 | Laboratory (71) |
| Leptin (ng/mL) | 11.3 – 63.7 | Literature (72) |
| Adiponectin (µg/mL) | 3.8 – 22.1 | Literature (51) |
| CRP (mg/L) | <8 | Literature (73) |

[1]Reference range not specific to non-pregnant women as values do not differ compared to pregravid.

**Table 4** Reference ranges for cardiometabolic biomarkers in late pregnancy for a healthy pregnant woman.

| Analyte | Reference Range in Late Pregnancy | Citation |
|---|---|---|
| Glucose (mmol/L) | 4.1 – 4.9 | Literature (74) |
| Insulin (pmol/L) | 48.06 – 86.39 | Literature (70) |
| Total Cholesterol (mmol/L) | 12.2 – 19.4 | Laboratory (71) |
| HDL (mmol/L) | 2.7 – 4.8 | Laboratory (71) |
| LDL (mmol/L) | 5.6 – 12.4 | Laboratory (71) |
| Triglycerides (mmol/L) | 7.3 – 25.2 | Laboratory (71) |
| Leptin (ng/mL) | 11.1 – 65.7 | Literature (72) |
| Adiponectin (µg/mL) | 2.9 – 19.4 | Literature (51) |
| CRP (mg/L) | 0.4 – 8.1 | Laboratory (71) |

### C.2.2 Assessment of adverse outcomes

The assessed adverse maternal health outcomes include blood pressure, the incidence of gestational diabetes mellitus, the incidence of preeclampsia, and method of delivery. Blood pressure was measured at 12-17, 26-28, 36-38 weeks. Measurements were conducted using the blood pressure monitor Omron® HEM-757 on the left arm while the participant was seated. Gestational diabetes mellitus and preeclampsia diagnosis were

determined by self-report at 26-28 or 36-38 weeks after having been diagnosed by their primary care physician. The method of delivery was determined through medical chart review (59).

### C.2.3 Maternal lifestyle factors – nutrition and physical activity

Diet and physical activity measures were collected pre-randomization at 12-17 weeks, as well as after randomization at 26-28 weeks and 36-38 weeks. Nutrient and supplement intakes were assessed through a standard 3-day food record which covered two weekdays and one weekend day. The 3-day food records were analyzed for nutrient intake using Nutritionist Prom™ (Version 5.2, Axxya Systems, Stafford, TX, USA) and the Canadian Nutrient File (version 2015) (59). Physical activity and exercise behaviours were assessed using a SenseWear® armband tri-axis accelerometer (Model MF-SW; BodyMedia® Inc., Pittsburgh PA). Participants wore the armbands on the back of the upper left arm across three days, coinciding with when the 3-day food records were conducted. The accelerometry data were downloaded from the device to a computer program provided by the SenseWear company which analyzed the recorded data for energy expenditure, step count, metabolic equivalents, and sleep duration using SenseWear® Professional 8.1 Software (BodyMedia® Inc., Pittsburgh PA).

Demographic information collected at baseline prior to randomization were used as co-variates. Demographic information was self-reported data collected on a REDCap-based case report form at enrollment, prior to randomization (59).

### C.2.4 Statistical analysis

Statistical analyses were performed using SPSS 26.0 (IBM Corp, 2016, Armonk, NY, USA). Descriptive statistics were presented as mean and standard deviation for normally distributed variables or as median and quartiles (Q1 and Q3) for non-normally distributed variables. The normality of variables was evaluated using Shapiro-Wilk test. To compare baseline characteristics between study arms, unpaired t-test was used for normally distributed continuous data, Mann Whitney U test was used for non-normally distributed continuous data, and Fischer exact test was used for categorical data. To evaluate differences in cardiometabolic biomarkers at different timepoints across pregnancy, a Wilcoxon Signed-Rank test was used. To evaluate associations between pBMI and cardiometabolic biomarker concentrations during pregnancy, generalized estimating equations (GEE) were used (75). Generalized estimating equations were adjusted for age, ethnicity, parity, study site, study arm, energy expenditure, time spent doing moderate to vigorous physical activity, energy intake, and polyunsaturated fatty acid to saturated fatty acid ratio. Statistical significance for the one-way Shaprio Wilk test, unpaired t-test, Mann Whitney U test, Fisher exact test, GEE, and Wilcoxon Signed-Rank test were defined as a two-sided p value of $<0.05$.

To compare dietary and physical activity measures during pregnancy between study arms, as well as to assess associations between diet and physical activity measures and pBMI, GEE was used. For the comparison between treatment groups, GEE were adjusted for stratification variables (pBMI and study site). For assessing associations with pBMI, GEE were adjusted for age, ethnicity, parity, study site, and study arm. To evaluate

differences between different timepoints, Wilcoxon Signed-Rank test was used. Statistical significance for GEE, and Wilcoxon Signed-Rank test were defined as a two-sided p value of <0.05.

To compare the intervention effect on the change in body composition across pregnancy, GEE were used. Models were adjusted for stratification variables (pBMI, study site, and study arm). An additional analysis was conducted evaluating the intervention effect on body composition 6 months post-partum, using the results collected from dual energy x-ray absorptiometry. For this analysis, ANCOVA was used and adjusted for stratification variables. Statistical significance for GEE and ANCOVA was defined as two-sided p-value of <0.05.

In evaluating the association between the change in maternal adiposity and cardiometabolic biomarkers, a multivariable linear regression model was used. Models were adjusted for age, ethnicity, parity, energy expenditure, time spent doing moderate to vigorous physical activity, energy intake, and polyunsaturated fatty acid to saturated fatty acid ratio (PUFA:SFA).

In analyzing the association between maternal adiposity and risk of adverse outcomes, logistic regression was used. The risk of gestational diabetes and preeclampsia were not analysed due to limited number of cases. Similar to the previous analyses, both the change in adiposity and early adiposity were examined. The adjusted models for caesarean section and operative vaginal delivery included age, ethnicity, parity, week gestation at birth, and infant birth weight as covariates.

The co-variates used in the regression analyses were measured at 12-17 weeks, prior to randomization. Unadjusted models were adjusted for stratification variables (pBMI, study site, and study arm). Normality of dependent variables for linear regression analyses were evaluated using normal p-p plot of regression standardized residuals and transformed accordingly (Table 5). For the multivariable and logistic regression, statistical significance was defined as a two-sided p-value of <0.05.

**Table 5** List of variables included in statistical analyses.

| Variable | Type | Transformation |
|---|---|---|
| Age | Continuous | None |
| Ethnicity | Categorical | None |
| Parity | Categorical | None |
| Average energy expenditure | Continuous | None |
| Average energy intake | Continuous | None |
| Time spent doing moderate to vigorous physical activity | Continuous | None |
| Sum of Skinfolds | Continuous | None |
| Percent body fat estimated by SFT | Continuous | None |
| Percent body fat estimated by BIA | Continuous | None |
| Pre-pregnancy body mass index | Continuous | None |
| Glucose | Continuous | None |
| Insulin | Continuous | Logarithmic |
| Total Cholesterol | Continuous | None |
| HDL | Continuous | None |
| LDL | Continuous | None |
| Triglycerides | Continuous | Square Root |
| Leptin | Continuous | Logarithmic |
| Adiponectin | Continuous | None |
| CRP | Continuous | Logarithmic |
| Systolic blood pressure (SBP) | Continuous | None |
| Diastolic blood pressure (DBP) | Continuous | None |

# CHAPTER 3

# COMPARATIVE ANALYSIS OF ADIPOSITY MEASURES

# CHAPTER 3 – RESULTS OF COMPARATIVE ANALYSIS OF ADIPOSITY MEASURES

## 3.1 PARTICIPANT DEMOGRAPHICS

Of the 241 women enrolled in the Be Healthy in Pregnancy study, 181 had complete data at all three visit time points for SFT measurements and BIA. Most participants were identified as of European descent, well-educated (completed a post-secondary degree), and had a household income greater than $75,000 (Table 6). In the analyzed sample, 40.9% of participants were categorized by body mass index (BMI) as being overweight/obese at the start of their pregnancy (Table 6).

**Table 6** Baseline demographic characteristics of study participants included in comparative analysis of adiposity measures.

| **Maternal characteristics** | ***N* = 181** |
|---|---|
| Gestational age at enrollment (wk) median (Q1, Q3) | 13.4 (12.6, 14.6) |
| Maternal age (yr) mean ± SD | 31.6 ± 4.0 |
| Pre-pregnancy BMI (kg/m$^2$) N (%) | |
| Underweight <18.5 | 2 (1.1) |
| Normal weight 18.5 – 24.9 | 105 (58.0) |
| Overweight 25 – 29.9 | 48 (26.5) |
| Obese ≥30 | 26 (14.4) |
| Ethnicity, N (%) | |
| European descent | 157 (86.7) |
| Mixed | 9 (5.0) |
| Other | 10 (5.5) |
| Household income, N (%) | |
| Household income ≥ $75000 | 131 (72.4) |
| Unknown/prefer not to answer | 8 (4.4) |
| Highest level of education, N (%) | |
| Tertiary | 172 (96.7) |
| Unknown/prefer not to answer | 4 (2.2) |
| Parity, N (%) | |
| 0 | 87 (48.1) |
| 1+ | 93 (51.4) |
| Unknown/prefer not to answer | 1 (0.5) |

### 3.2 Comparison of estimates of percent body fat across pregnancy

In early pregnancy, %BF by BIA and by SFT demonstrated good agreement, with BIA measures on average 1.8% greater than SFT (Figure 1). This is further supported by the strong Pearson's correlation and moderate Lin's concordance coefficient between measures (Table 7).

**Figure 1** Comparison of %BF estimated by BIA and SFT at 12-17 weeks gestation. Dashed line is the mean difference (Bias). Dotted lines are the limits of agreement (mean difference ± 1.96SD); *n* = 181.

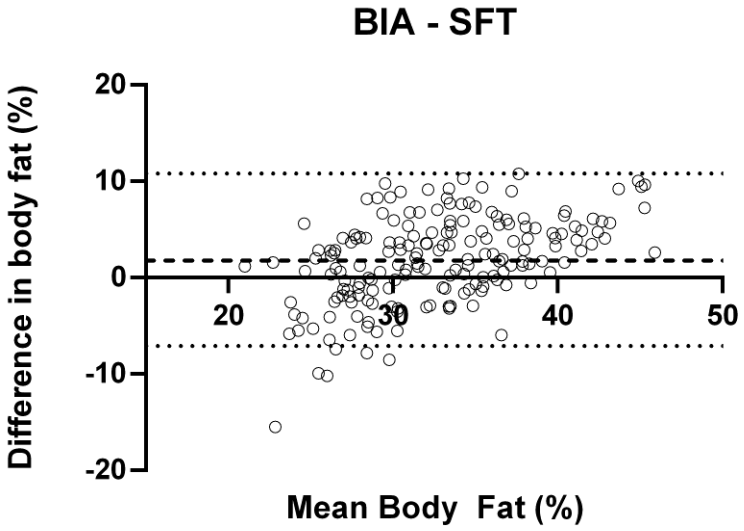



**Table 7** Strength of association between %BF estimated by BIA and SFT at different gestational stages.

| Gestational Stage | Pearson's Coefficient | *p* | Lin's Concordance Coefficient (95% CI) |
|---|---|---|---|
| Early Pregnancy | 0.75 | <0.001 | 0.67 (0.61, 0.72) |
| Mid Pregnancy | 0.70 | <0.001 | 0.52 (0.46, 0.58) |
| Late Pregnancy | 0.61 | <0.001 | 0.31 (0.26, 0.36) |

As pregnancy progressed, the agreement between % BF measures became lower with BIA measures of 4.1% (Figure 2) and 7.1% (Figure 3) greater than SFT in the second and

third trimester, respectively. Lin's concordance coefficient also demonstrated lower agreement in both second and third trimesters (Table 7). However, correlation between measures remained strong in later gestational stages (Table 7). At all three stages of pregnancy, the Bland-Altman plots demonstrated an upward trend, indicating that there is a greater difference between measures at increasing average %BF (Figure 1-3). Across pregnancy, mean difference between measures in %BF increased significantly ($p <$ 0.001).

**Figure 2** Comparison of %BF estimated by BIA and SFT at 26-28 weeks gestation. Dashed line is the mean difference (Bias). Dotted lines are the limits of agreement (mean difference ± 1.96SD); $n$ = 181.

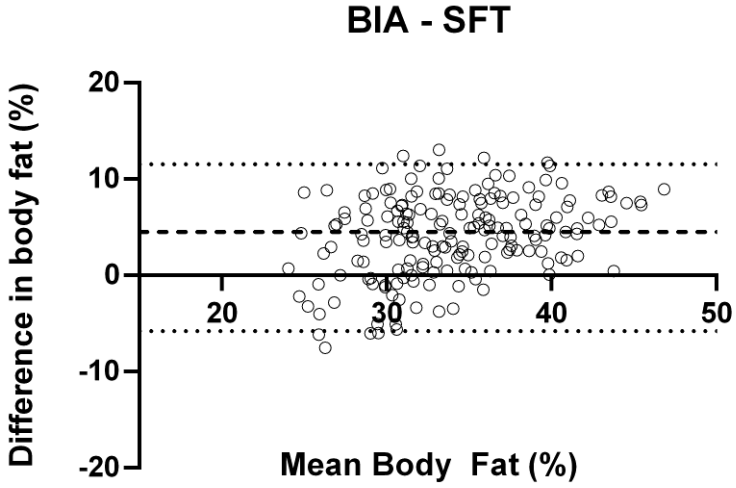
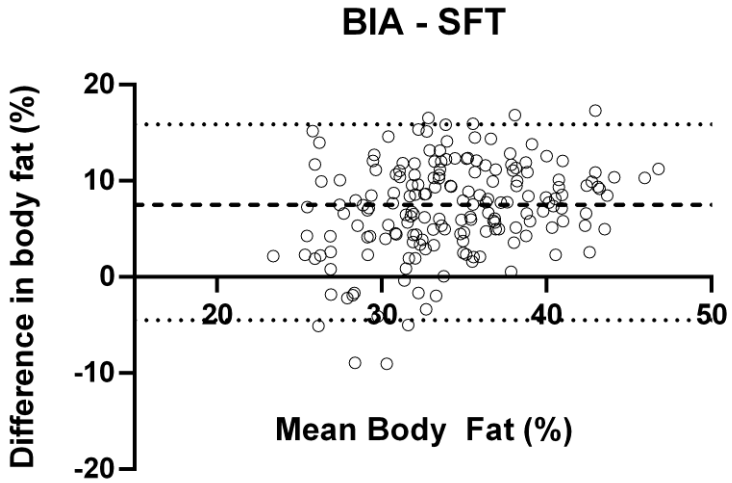


**Figure 3** Comparison of %BF estimated by BIA and SFT at 36-38 weeks gestation. Dashed line is the mean difference (Bias). Dotted lines are the limits of agreement (mean difference ± 1.96SD); *n* = 181.




At 6 months post-partum, BIA and SFT again demonstrated good agreement with BIA measures being 1.7% greater than %BF estimated by SFT, which was similar to findings in early pregnancy (Figure 4). As well, there was a strong correlation and moderate Lin's concordance coefficient between measures (Table 8).

In evaluating agreement between both BIA and SFT with DXA, BIA had good agreement with DXA, with DXA being on average 0.5% greater than BIA measures (Figure 5). This strong agreement is also demonstrated by the strong correlation and moderate Lin's concordance coefficient (Table 8). In contrast, the agreement between DXA and SFT was weaker compared to BIA and DXA, with DXA measures being on average 2.2% greater than SFT (Figure 6). However, DXA and SFT measures demonstrated a strong correlation and moderate Lin's concordance coefficient (Table 8).

**Figure 4** Comparison of %BF estimated by BIA and SFT at 6 months postpartum. Dashed line is the mean difference (Bias). Dotted lines are the limits of agreement (mean difference ± 1.96SD); *n* = 141.

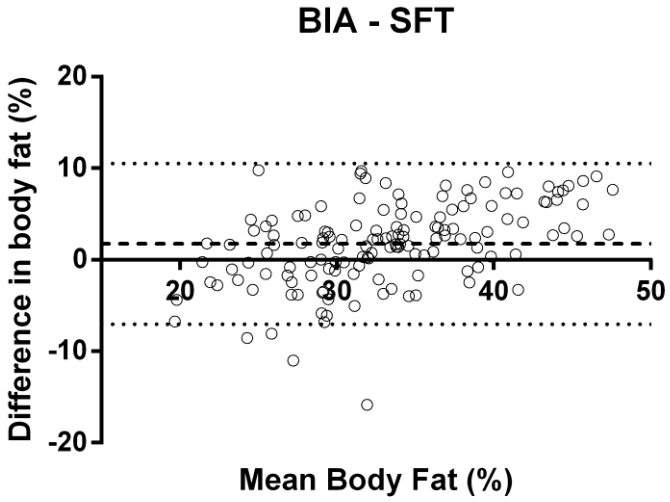



**Table 8** Strength of association between estimates of %BF at 6 months postpartum.

| Adiposity tools being compared | Pearson's Coefficient | *p* | Lin's Concordance Coefficient (95% CI) |
|---|---|---|---|
| BIA vs. SFT | 0.81 | <0.001 | 0.76 (0.71, 0.80) |
| DXA vs. BIA | 0.83 | <0.001 | 0.82 (0.78, 0.86) |
| DXA vs. SFT | 0.86 | <0.001 | 0.79 (0.75, 0.83) |

**Figure 5** Comparison of %BF estimated by DXA and BIA at 6 months postpartum. Dashed line is the mean difference (Bias). Dotted lines are the limits of agreement (mean difference ± 1.96SD); *n* = 141.

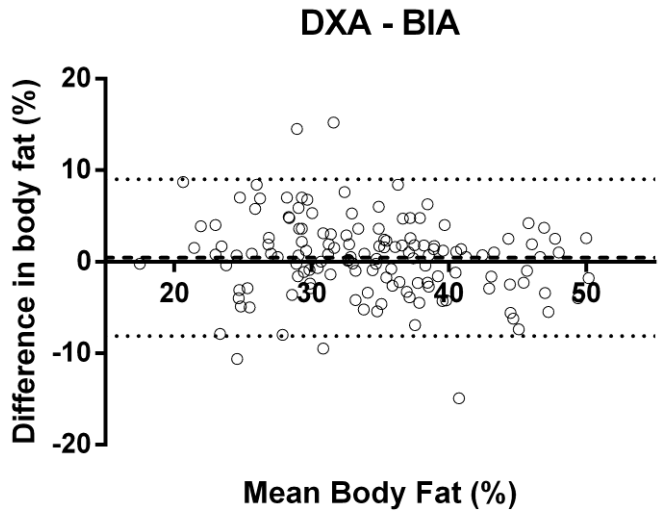
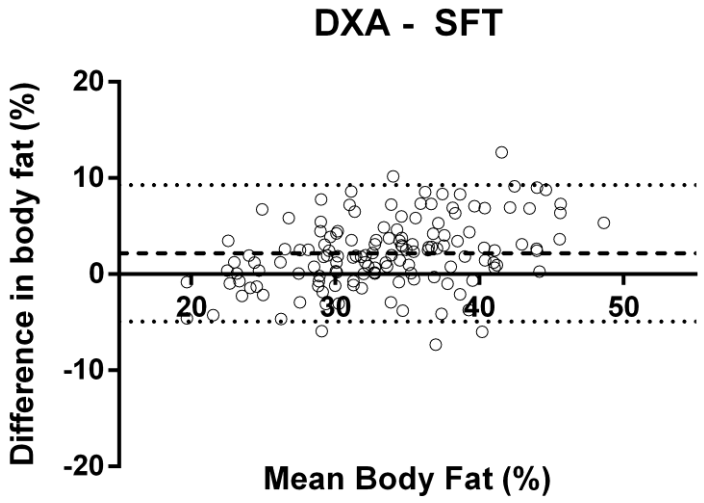



**Figure 6** Comparison of %BF estimated by DXA and SFT at 6 months postpartum. Dashed line is the mean difference (Bias). Dotted lines are the limits of agreement (mean difference ± 1.96SD); *n* = 141.




### 3.3 Adiposity Trends Across Pregnancy

Adiposity was positively associated with advancing gestational age when measured by sum of SFT (r = 0.12, $p = 0.0043$) (Figure 7) and %BF by BIA (r = 0.25, $p < 0.001$)

(Figure 8). In contrast, %BF calculated from SFT was not associated with gestational age (r = -0.08, $p$ = 0.06) (Figure 9). There were significant differences between medians for both SFT measures and averages for %BF by BIA, for all visit time points (Table 9). Associations between adiposity and gestational age stratified by pBMI is in Appendix 2.

**Figure 7** Relationship between sum of SFT and gestational age. r = 0.1191, $p$ = 0.004, $n$ = 191.

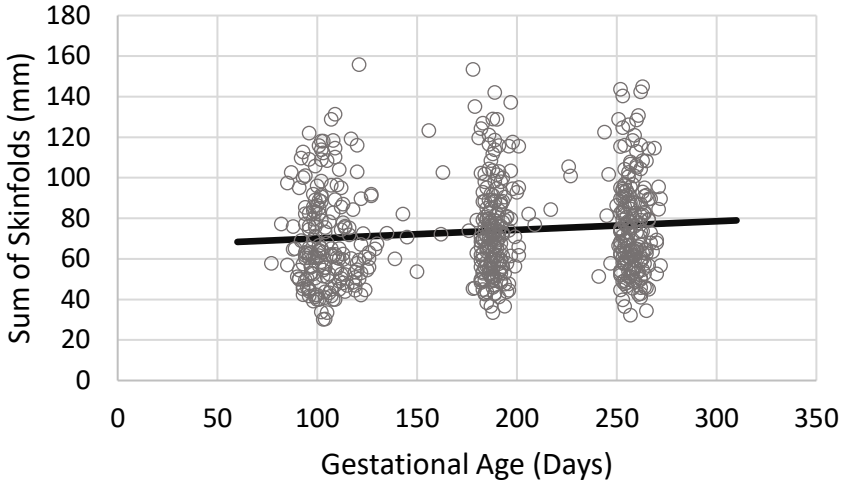



**Figure 8** Relationship between %BF by SFT and gestational age. r = -0.0783, $p$ = 0.061, $n$ = 191.

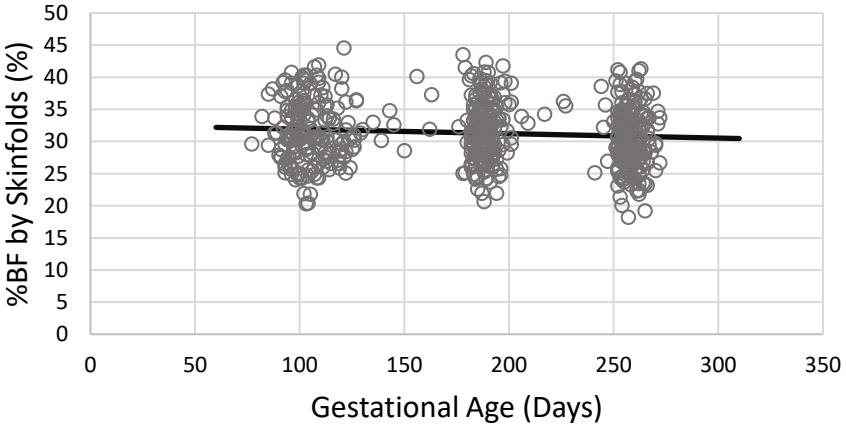


**Figure 9** Relationship between %BF by BIA and gestational age. r = 0.2462, $p < 0.001$, $n = 182$.

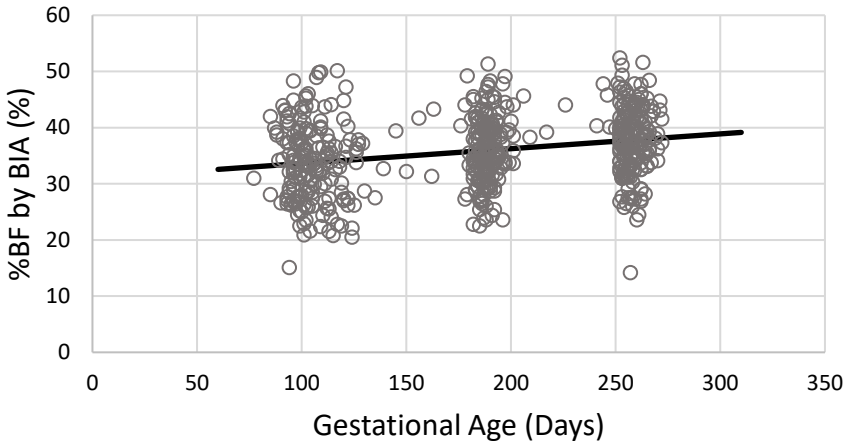



**Table 9** Adiposity measures at early, mid, and late pregnancy.

| Measure | Early Pregnancy | Mid Pregnancy | Late Pregnancy | Overall p |
|---|---|---|---|---|
| Sum of SFT (mm)[1] | 63.1 (52.3, 85.0) | 71.3 (56.3, 88.3) | 73.5 (58.6, 90.9)[3] | 0.009 |
| %BF by SFT (%)[2] | 31.6 ± 4.8 | 31.8 ± 4.6 | 30.6 ± 4.7[4] | 0.022 |
| %BF by BIA (%)[2] | 33.6 ± 6.9 | 36.1 ± 5.9[3] | 37.7 ± 6.1[3,4] | <0.001 |

[1]Reported as median (Q1, Q3); difference between time points evaluated using Friedman test and Dunn's multiple comparisons test
[2]Reported as mean±SD; difference between time points evaluated using repeated measures one-way ANOVA and Tukey's multiple comparisons test
[3]Outcome significantly different from early pregnancy
[4]Outcome significantly different from mid pregnancy

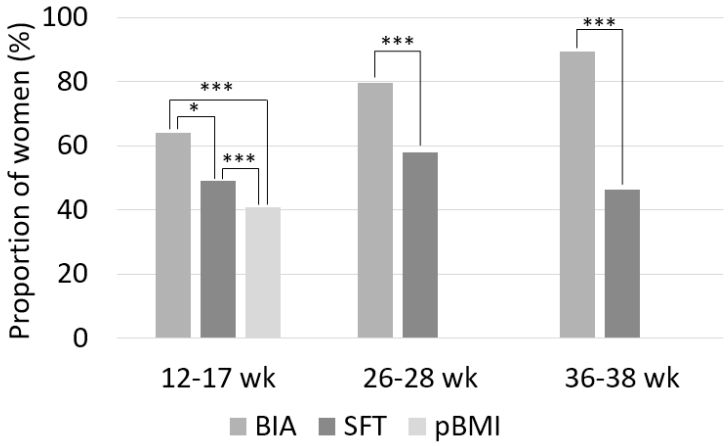
### 3.4 Categorization of normal or overweight status by body fat measures

In evaluating the discrimination power to categorize women as normal weight or overweight/obese, the c-statistic reveals strong concordance when comparing pBMI with either %BF estimated by SFT (c = 0.903 [95%CI = 0.859, 0.947]) or with BIA (c = 0.929 [95%CI = 0.890, 0.968]). However, a greater number of women were categorized as

overweight/obese in early pregnancy by both %BF measures ($p < 0.001$) when compared to pBMI (Figure 10).

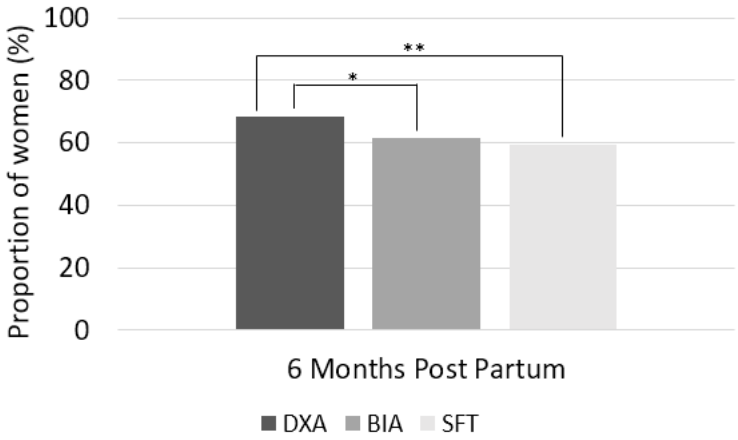
A greater proportion of women were categorized as overweight/obese by BIA in comparison to %BF estimated by SFT in the first ($p < 0.05$), second ($p < 0.001$), and third ($p < 0.001$) trimesters (Figure 10). At 6 months postpartum, there was no significant difference ($p = 0.64$) in the proportion of women categorized as overweight/obese by %BF estimated by BIA when compared to %BF estimated by SFT (Figure 11). However, %BF estimated by DXA categorized significantly more women as overweight/obese when compared to %BF estimated by BIA ($p < 0.05$) and %BF estimated by SFT ($p < 0.001$) (Figure 11).

**Figure 10** Proportion of participants categorized as overweight/obese based on %BF measures or pBMI at early, mid, and late pregnancy; $n = 181$.




*$p$ value <0.05 (McNemar's test); ***$p$ value <0.0001 (McNemar's test).

**Figure 11** Proportion of participants categorized as overweight/obese based on %BF measures by 3 methods at 6 months post-partum; *n* = 141.




**p* value <0.05 (McNemar's test); ***p* value <0.001 (McNemar's test).

# CHAPTER 4

# IMPACT OF INTERVENTION STUDY ON CLINICAL OUTCOMES

# CHAPTER 4 – IMPACT OF INTERVENTION STUDY ON CLINICAL OUTCOMES

## 4.1 DIET AND EXERCISE INTERVENTION IMPACT ON OUTCOMES

### 4.1.1 BASELINE CHARACTERISTICS

From the 241 women enrolled, 192 had either BIA measurements or SFT measurements taken at all study visits and were included in the treatment analysis. Among this sub-sample, there were no significant differences between treatment groups for any baseline characteristics (Table 10). This sub-sample is biased with a lower median pBMI and a different distribution of pBMI categories compared to the women not included in this analysis (Appendix 3).

**Table 10** Baseline demographic characteristics of study participants with complete BIA or SFT measurements by treatment group

| Maternal characteristics | Intervention N = 96 | Control N = 96 | *p*[1] |
|---|---|---|---|
| Gestational age at enrollment (wk) median (Q1, Q3) | 14.7(13.9, 16.0) | 14.6 (13.6, 16.1) | 0.270 |
| Maternal age (yr) mean ± SD | 31.6 ± 3.9 | 31.5 ± 4.0 | 0.812 |
| Pre-pregnancy BMI (kg/m$^2$) median (Q1, Q3) | 24.4 (22.1, 27.5) | 24.0 (21.6, 27.0) | 0.447 |
| Underweight N (%) | 2 (2.1) | 1 (1.0) | 0.953 |
| Normal weight N (%) | 53 (55.2) | 56 (58.3) | |
| Overweight N (%) | 26 (27.1) | 25 (26.0) | |
| Obese N (%) | 15 (15.6) | 14 (14.6) | |
| Ethnicity N (%) | | | 1.000 |
| European descent | 87 (90.6) | 86 (89.6) | |
| Mixed | 4 (4.2) | 5 (5.2) | |
| Other | 5 (5.2) | 5 (5.2) | |
| Household income N (%) | | | 0.222 |
| Household income ≥ $75000 | 76 (79.2) | 65 (67.7) | |
| Unknown/prefer not to answer | 2 (2.1) | 6 (6.3) | |
| Highest level of education N (%) | | | 0.497 |
| Tertiary | 90 (93.8) | 88 (91.7) | |
| Unknown/prefer not to answer | 3 (3.1) | 2 (2.1) | |
| Parity N (%) | | | 1.000 |
| 0 | 47 (49.0) | 47 (49.0) | |
| 1+ | 49 (51.0) | 48 (50.0) | |
| Unknown/prefer not to answer | 0 (0.0) | 1 (1.0) | |

[1]To assess differences between study arm, unpaired t-test was used for normal continuous data, Mann Whitney U test was used for non-normal continuous data, Fisher's exact test was used for categorical data.

### 4.1.2 Change in adiposity across pregnancy

The change in sum of SFT, change %BF estimated by SFT, and change in %BF estimated by BIA across pregnancy was similar for intervention and control groups (Table 11).

**Table 11** Treatment effect on change in sum of SFT across pregnancy[1]

| Outcome[2] | B (95% CI)[2] | *p* |
|---|---|---|
| Sum of SFT (mm) | -1.4 (-5.3, 2.5) | 0.495 |
| %BF by SFT (%) | -0.3 (-1.1, 0.5) | 0.455 |
| %BF by BIA (%) | 0.3 (-0.8, 1.3) | 0.604 |

[1]GEE adjusted for pBMI and study site; intervention n = 96, control n = 95.
[2]B = unstandardized beta coefficient

### 4.1.3 Body composition at 6 months postpartum

There was no significant intervention effect at 6 months post partum for bone mass, FM, lean mass, %BF, and total mass (Table 12).

**Table 12** Treatment effect on body composition measures by DXA at 6 months postpartum.[1]

| Body Composition Measure | Intervention *N* = 71 | Control *N* = 72 | *p* |
|---|---|---|---|
| Fat Mass (g) | 26040 | 26143 | 0.701 |
| Lean Mass (g) | 44768 | 45189 | 0.579 |
| Percent Body Fat (%) | 34.6 | 34.5 | 0.292 |
| Total Mass (kg) | 71.8 | 73.6 | 0.121 |

[1]ANCOVA adjusted for pBMI and study site

### 4.1.4 Dietary and physical activity measures across pregnancy

At baseline, records of self-selected diet components and physical activity by accelerometry were similar between groups subsequently randomized to treatment and control (Table 13). Following randomization into either treatment or control, most diet and physical activity measures did not differ between study arms during pregnancy, except for protein intake which was significantly greater in the intervention group compared to the control group (Table 14).

**Table 13** Baseline dietary and physical activity measures in early pregnancy. Values presented as mean ± SD.[1]

| Outcome | Intervention *n* = 111 - 117 | Control *n* = 107 - 115 | *p* |
|---|---|---|---|
| Energy Expenditure (kcal/day) | 2085 ± 312 | 2084 ± 357 | 0.568 |
| Energy Intake (kcal/day) | 2147.645 ± 490.777 | 2148.548 ± 538.706 | 0.709 |
| Moderate to vigorous physical activity (minutes/day) | 48 ± 33 | 51 ± 38 | 0.790 |
| Step count (steps/day) | 5246 ± 2924 | 5375 ± 2538 | 0.758 |
| Fat Intake (g) | 80.728 ± 25.088 | 80.984 ± 25.237 | 0.959 |
| Carbohydrate Intake (g) | 276.865 ± 67.494 | 277.320 ± 82.818 | 0.571 |
| Protein Intake (g) | 84.6 ± 22.5 | 87.5 ± 23.8 | 0.260 |
| PUFA:SFA | 0.413 ± 0.273 | 0.429 ± 0.212 | 0.498 |

[1]ANCOVA adjusted for pBMI and study site.

**Table 14** Average treatment effect on dietary and physical activity measures at mid and late pregnancy following randomization. Values presented as mean ± SD.[1]

| Outcome | *n* | B (95% CI) | *p* |
|---|---|---|---|
| Energy Expenditure (kcal/day) | 202 | -6 (-98, 86) | 0.898 |
| Energy Intake (kcal/day) | 201 | 41.124 (-91.220, 173.468) | 0.543 |
| Moderate to vigorous physical activity (minutes/day) | 202 | -4 (-15, 7) | 0.500 |
| Step Count (steps/day) | 185 | 136 (437, 992) | 0.756 |
| Fat Intake (g) | 201 | -4.322 (-11.400, 2.755) | 0.231 |
| Carbohydrate Intake (g) | 201 | 0.275 (-19.672, 20.222) | 0.978 |
| Protein Intake (g) | 201 | 20.153 (12.994, 27.311) | <0.001 |
| PUFA:SFA | 201 | 0.015 (-0.055, 0.084) | 0.680 |

[1]GEE adjusted for pBMI and study site.

By analysis of data across treatment groups, the average effect of pBMI on diet and physical activity measures across pregnancy was assessed. Energy, fat and carbohydrate intakes, and polyunsaturated fatty acid to saturated fatty acid consumption was not associated with pBMI (Table 15). Energy expenditure was significantly greater with increasing pBMI during pregnancy (Table 15). There was also a decrease in time spent doing moderate to vigorous physical activity with increasing pBMI during pregnancy (Table 15).

**Table 15** Average effect of pBMI on dietary and physical activity measures at early, mid, and late pregnancy. Values are shows as mean ± SD.[1]

| **Measures** | ***n*** | **B (95% CI)** | ***p*** |
|---|---|---|---|
| Energy Expenditure (kcal/day) | 232 | 36 (27, 45) | <0.001 |
| Energy Intake (kcal/day) | 230 | 7.834 (-5.940, 21.607) | 0.265 |
| Moderate to vigorous physical activity (minutes/day) | 232 | -2 (-2, -1) | <0.001 |
| Step count (steps/day) | 183 | 2 (-75, 78) | 0.970 |
| Fat Intake (g) | 230 | 0.246 (-0.458, 0.951) | 0.493 |
| Carbohydrate Intake (g) | 230 | 1.307 (-0.919, 3.532) | 0.250 |
| Protein Intake (g) | 230 | -0.154 (-0.779, 0.472) | 0.630 |
| PUFA:SFA | 230 | -0.002 (-0.007, 0.003) | 0.505 |

[1]GEE adjusted for age, ethnicity, parity, study site, and study arm.

## 4.3 Cardiometabolic outcomes

### 4.3.1 Cardiometabolic status across pregnancy

Of the 192 women analyzed by treatment group (Section 4.1), 183 participants provided a fasted blood sample at early and late pregnancy. This sub-sample has similar

baseline characteristics as presented in Table 10 in section 4.1.1 with 43.2% of women categorized as overweight/obese and a mean pBMI of 31.6 ± 3.9.

Median concentrations for glucose, leptin, adiponectin, and CRP were within reference ranges outlined in Table 3 and Table 4 for early and late pregnancy, respectively (Table 17). Median concentrations for total cholesterol, HDL, LDL, and triglycerides were lower than reference ranges for early and late pregnancy as outlined in Table 3 and Table 4, respectively (Table 16). Median insulin concentrations were lower than reference ranges for early pregnancy as outlined in Table 3 (Table 16). Most cardiometabolic biomarkers rose from early to late pregnancy except for glucose and adiponectin which decreased and HDL which remained stable (Table 16).

**Table 16** Cardiometabolic biomarkers concentrations in both early and late pregnancy. Values presented as median (Q1, Q3).

| Biomarker | Early Pregnancy[1] | Late Pregnancy[1] | *p*[2] |
|---|---|---|---|
| Glucose (mmol/L) | 4.7 (4.5, 5.1) | 4.6 (4.3, 4.9) | <0.001 |
| Insulin (pmol/L) | 30.53 (19.59, 48.47) | 50.67 (32.19, 79.80) | <0.001 |
| Total Cholesterol (mmol/L) | 5.26 (4.66,5.95) | 6.91 (6.27, 8.08) | <0.001 |
| Triglycerides (mmol/L) | 1.19 (0.94, 1.50) | 2.49 (2.08, 3.02) | <0.001 |
| HDL (mmol/L) | 1.82 (1.59, 2.03) | 1.82 (1.55, 2.11) | 0.279 |
| LDL (mmol/L) | 2.83 (2.47, 3.37) | 3.89 (3.32, 4.71) | <0.001 |
| Leptin (ng/mL) | 22.22 (12.86, 39.44) | 30.04 (15.28, 52.20) | <0.001 |
| Adiponectin (µg/mL) | 8.73 (5.98, 10.83) | 6.80 (5.00, 9.21) | <0.001 |
| CRP (mg/L) | 4.86 (2.26, 8.08) | 4.39 (2.18, 7.37) | 0.032 |
| SBP | 108 (102, 116) | 114 (105, 120) | <0.001 |
| DBP | 68 (63, 73) | 72 (68, 78) | <0.001 |

[1]*n* = 171 – 182.
[2]Wilcoxon signed ranks test

During pregnancy, greater pBMI was associated with significantly higher mean glucose, insulin, triglyceride, leptin, and CRP concentrations, as well as significantly lower HDL and adiponectin concentrations (Table 17). Increasing pBMI was associated

with a reduced change in total cholesterol and LDL across pregnancy (Table 18). As well, increasing pBMI was associated with an increased change in adiponectin across pregnancy (Table 18).

**Table 17** Average effect of pBMI on cardiometabolic biomarker concentrations during pregnancy. Values presented as mean ± SD.[1]

| Biomarker | B (95% CI) | *p* |
|---|---|---|
| Glucose (mmol/L) | 0.041 (0.030, 0.051) | <0.001 |
| Insulin (pmol/L) | 3.222 (2.106, 4.338) | <0.001 |
| Total Cholesterol (mmol/L) | -0.026 (-0.059, 0.008) | 0.134 |
| Triglycerides (mmol/L) | 0.038 (0.020, 0.055) | <0.001 |
| HDL (mmol/L) | -0.029 (-0.044, -0.015) | <0.001 |
| LDL (mmol/L) | -0.013 (-0.040, 0.015) | 0.366 |
| Leptin (ng/mL) | 3.604 (2.610, 4.598) | <0.001 |
| Adiponectin (µg/mL) | -0.302 (-0.406, -0.197) | <0.001 |
| CRP (mg/L) | 0.269 (0.049, 0.489) | 0.017 |

[1]GEE adjusted for age, ethnicity, parity, study site, study arm, energy expenditure, time spent doing moderate to vigorous physical activity, energy intake, PUFA:SFA.

**Table 18** Average effect of pBMI on change in cardiometabolic biomarker concentrations across pregnancy. Values presented as mean ± SD.[1]

| Biomarker | B (95% CI) | *p* |
|---|---|---|
| Glucose (mmol/L) | 0.011 (-0.005, 0.028) | 0.185 |
| Insulin (pmol/L) | 0.626 (-0.897, 2.149) | 0.421 |
| Total Cholesterol (mmol/L) | -0.055 (-0.085, -0.024) | <0.001 |
| Triglycerides (mmol/L) | -0.002 (-0.028, 0.024) | 0.878 |
| HDL (mmol/L) | 0.008 (-0.002, 0.018) | 0.100 |
| LDL (mmol/L) | -0.066 (-0.088, -0.043) | <0.001 |
| Leptin (ng/mL) | 0.039 (-0.556, 0.634) | 0.898 |
| Adiponectin (µg/mL) | 0.130 (0.066, 0.193) | <0.001 |
| CRP (mg/L) | 0.004 (-0.185, 0.193) | 0.966 |

[1]GEE adjusted for age, ethnicity, parity, study site, study arm, energy expenditure, time spent doing moderate to vigorous physical activity, energy intake, PUFA:SFA.

### 4.3.2 CARDIOMETABOLIC STATUS ASSOCIATED WITH CHANGE IN ADIPOSITY

In evaluating the association between change in sum of SFT across pregnancy and cardiometabolic biomarkers, insulin and leptin were significantly associated in both the unadjusted and adjusted models (Table 19).

**Table 19** Association of change in sum of SFT across pregnancy with cardiometabolic biomarkers.

| Outcome | Unadjusted | | Adjusted[1] | |
|---|---|---|---|---|
| | B (95% CI) | *p* | B (95% CI) | *p* |
| Glucose (mmol/L) | -0.001 (-0.006, 0.003) | 0.549 | -0.001 (-0.006, 0.005) | 0.843 |
| Insulin (pmol/L) | 0.003 (0.001, 0.006) | 0.020 | 0.003 (0.000, 0.006) | 0.031 |
| HDL (mmol/L) | 0.002 (-0.003, 0.007) | 0.420 | 0.002 (-0.003, 0.007) | 0.531 |
| LDL (mmol/L) | -0.001 (-0.012, 0.011) | 0.922 | 0.001 (-0.011, 0.013) | 0.877 |
| Triglycerides (mmol/L) | -0.001 (-0.004, 0.001) | 0.354 | -0.001 (-0.004, 0.002) | 0.489 |
| Total Cholesterol (mmol/L) | 0.000 (-0.014, 0.014) | 0.992 | 0.002 (-0.013, 0.016) | 0.821 |
| Leptin (ng/mL) | 0.005 (0.002, 0.008) | 0.003 | 0.005 (0.002, 0.008) | 0.004 |
| Adiponectin (µg/mL) | -0.015 (-0.047, 0.017) | 0.356 | -0.011 (-0.434, 0.022) | 0.524 |
| CRP (mg/L) | 0.000 (-0.004, 0.004) | 0.810 | -0.001 (-0.005, 0.003) | 0.701 |
| SBP (mmHg) | 0.040 (-0.075, 0.154) | 0.493 | 0.026 (-0.093, 0.145) | 0.670 |
| DBP (mmHg) | 0.024 (-0.067, 0.114) | 0.606 | 0.014 (-0.081, 0.109) | 0.765 |

[1]Adjusted for age, ethnicity, parity, energy expenditure, moderate to vigorous physical activity, energy intake, PUFA:SFA.

Similar to SFT, change in %BF estimated by SFT across pregnancy was significantly associated with insulin and leptin in both the unadjusted and adjusted models (Table 20).

**Table 20** Association of change in %BF by SFT across pregnancy with cardiometabolic biomarkers.[1]

| Outcome | Unadjusted | | Adjusted[1] | |
|---|---|---|---|---|
| | B (95% CI) | *p* | B (95% CI) | *p* |
| Glucose (mmol/L) | -0.005 (-0.030, 0.020) | 0.675 | 0.000 (-0.027, 0.027) | 0.983 |
| Insulin (pmol/L) | 0.017 (0.004, 0.031) | 0.011 | 0.018 (0.004, 0.032) | 0.013 |
| HDL (mmol/L) | 0.005 (-0.019, 0.029) | 0.684 | 0.002 (-0.024, 0.028) | 0.880 |
| LDL (mmol/L) | -0.013 (-0.072, 0.046) | 0.670 | -0.004 (-0.067, 0.058) | 0.894 |
| Triglycerides (mmol/L) | -0.008 (-0.021, 0.005) | 0.242 | -0.006 (-0.019, 0.008) | 0.423 |
| Total Cholesterol (mmol/L) | -0.017 (-0.088, 0.053) | 0.628 | -0.008 (-0.083, 0.067) | 0.836 |
| Leptin (ng/mL) | 0.029 (0.012, 0.046) | 0.001 | 0.029 (0.011, 0.047) | 0.002 |
| Adiponectin (µg/mL) | -0.059 (-0.222, 0.103) | 0.472 | -0.043 (-0.211, 0.126) | 0.617 |
| CRP (mg/L) | -0.004 (-0.024, 0.016) | 0.776 | -0.007 (-0.029, 0.015) | 0.527 |
| SBP (mmHg) | 0.207 (-0.382, 0.795) | 0.489 | 0.105 (-0.517, 0.727) | 0.739 |
| DBP (mmHg) | 0.070 (-0.396, 0.536) | 0.767 | 0.008 (-0.489, 0.505) | 0.975 |

[1] Adjusted for age, ethnicity, parity, energy expenditure, moderate to vigorous physical activity, energy intake, polyunsaturated fat: saturated fat.

In evaluating the association between change in %BF estimated by BIA across pregnancy and cardiometabolic biomarkers, HDL was positively associated with %BF while triglycerides and SBP were negatively associated with %BF in both the unadjusted and adjusted models (Table 21). As well, DBP was negatively associated with change in %BF estimated by BIA, however this was only in the unadjusted model. These results were not consistent with the associations found between the change in SFT measures of adiposity across pregnancy and cardiometabolic biomarkers (Table 19 and Table 20).

**Table 21** Association of change in %BF by BIA across pregnancy with cardiometabolic biomarkers.

| Outcome | Unadjusted | | Adjusted[1] | |
|---|---|---|---|---|
| | B (95% CI) | *p* | B (95% CI) | *p* |
| Glucose (mmol/L) | -0.009 (-0.029, 0.011) | 0.373 | -0.008 (-0.029, 0.014) | 0.474 |
| Insulin (pmol/L) | 0.000 (-0.012, 0.011) | 0.974 | -0.002 (-0.014, 0.009) | 0.704 |
| HDL (mmol/L) | 0.022 (0.002, 0.041) | 0.027 | 0.020 (0.000, 0.041) | 0.052 |
| LDL (mmol/L) | -0.026 (-0.076, 0.023) | 0.293 | -0.015 (-0.067, 0.037) | 0.562 |
| Triglycerides (mmol/L) | -0.017 (-0.027, -0.006) | 0.002 | -0.015 (-0.026, -0.004) | 0.007 |
| Total Cholesterol (mmol/L) | -0.020 (-0.078, 0.037) | 0.483 | -0.007 (-0.067, 0.054) | 0.826 |
| Leptin (ng/mL) | 0.009 (-0.005, 0.024) | 0.193 | 0.006 (-0.009, 0.021) | 0.408 |
| Adiponectin (µg/mL) | -0.013 (-0.146, 0.120) | 0.844 | 0.015 (-0.121, 0.151) | 0.831 |
| CRP (mg/L) | 0.014 (-0.005, 0.030) | 0.107 | 0.011 (-0.007, 0.028) | 0.235 |
| SBP (mmHg) | -0.753 (-1.221, -0.286) | 0.002 | -0.672 (-1.164, -0.181) | 0.008 |
| DBP (mmHg) | -0.393 (-0.761, -0.026) | 0.036 | -0.326 (-0.714, 0.062) | 0.099 |

[1] Adjusted for age, ethnicity, parity, energy expenditure, moderate to vigorous physical activity, energy intake, polyunsaturated fat: saturated fat.

### 4.4 Change in adiposity and adverse outcomes

Greater change in adiposity by any of the three measures was not associated with increased risk of adverse outcomes of caesarean section or operative vaginal delivery (Table 22-24). Gestational diabetes mellitus and preeclampsia were not included in this analysis due to having too few cases.

**Table 22** Association of change in sum of SFT across pregnancy with adverse outcomes

| **Outcome** | **Unadjusted** | | | **Adjusted[1]** | | |
|---|---|---|---|---|---|---|
| | *n* (Cases) | OR (95% CI) | *p* | *n* (Cases) | OR (95% CI) | *p* |
| Caesarean Section | 179 (38) | 0.977 (0.952, 1.004) | 0.092 | 177 (37) | 0.979 (0.951, 1.007) | 0.138 |
| Operative Vaginal Delivery | 179 (18) | 1.011 (0.977, 1.047) | 0.532 | 177 (18) | 1.014 (0.980, 1.050) | 0.420 |

[1]Adjusted for age, ethnicity, parity, week gestation at birth, infant weight at birth.

**Table 23** Association of change in %BF by SFT across pregnancy with adverse outcomes

| **Outcome** | **Unadjusted** | | | **Adjusted[1]** | | |
|---|---|---|---|---|---|---|
| | *n* (Cases) | OR (95% CI) | *p* | *n* (Cases) | OR (95% CI) | *p* |
| Caesarean Section | 179 (38) | 0.887 (0.775, 1.017) | 0.086 | 177 (37) | 0.896 (0.775 1.035) | 0.136 |
| Operative Vaginal Delivery | 179 (18) | 1.084 (0.906, 1.298) | 0.376 | 177 (18) | 1.110 (0.920, 1.340) | 0.275 |

[1]Adjusted for age, ethnicity, parity, week gestation at birth, infant weight at birth.

**Table 24** Association of change in %BF by BIA across pregnancy with adverse outcomes

| **Outcome** | **Unadjusted** | | | **Adjusted[1]** | | |
|---|---|---|---|---|---|---|
| | *n* (Cases) | OR (95% CI) | *p* | *n* (Cases) | OR (95% CI) | *p* |
| Caesarean Section | 172 (36) | 1.005 (0.898, 1.125) | 0.931 | 170 (36) | 1.016 (0.905, 1.141) | 0.786 |
| Operative Vaginal Delivery | 172 (19) | 1.021 (0.880, 1.183) | 0.787 | 170 (19) | 1.033 (0.890, 1.199) | 0.671 |

[1]Adjusted for age, ethnicity, parity, week gestation at birth, infant weight at birth.

# CHAPTER 5

# DISCUSSION AND CONCLUSION

# CHAPTER 5 – DISCUSSION AND CONCLUSION

## 5.1 ADIPOSITY CHANGES IN PREGNANCY

Maternal FM is known to increase during pregnancy, then decline in the post partum (76), which is consistent with our findings as adiposity increased across pregnancy when evaluated as the sum of SF or %BF by BIA. A gain in total FM or %BF across pregnancy is a result of normal cardiometabolic adaptations that occur to ensure appropriate energy use and storage during pregnancy to meet the changing energy demands (34–37). However, external factors may also influence the amount of fat gained during pregnancy. Understanding how these specific factors influence maternal adiposity can guide potential lifestyle interventions for mediating fat gain during pregnancy.

***Nutrition+Exercise intervention:*** Increases in adiposity measured as %BF across pregnancy were not influenced by the nutrition+exercise intervention compared to standard care where women consumed their habitual self-selected diets. Neither was there a treatment effect for absolute measures of FM, lean mass, and bone mass at 6 months postpartum. Two lifestyle variables may explain the observed lack of an intervention effect. Despite energy intake being individually prescribed for the intervention group, energy intakes and expenditure were calculated to be similar between groups, resulting in similar energy balances across pregnancy. Further, women in the intervention met their projected energy requirements and given the similar energy balance, it can be assumed that the control group was meeting their requirements as well. Energy deficits are often accompanied by weight loss and fat loss in non-pregnant populations (77,78). However, incorporating energy deficits to mediate GWG or fat gain may not be appropriate due to

potential risks to the fetus (79–81). Changes in adiposity are also influenced by diet composition and quality (77,82–85). Thus, the lack of intervention effect might also be attributed to the similar carbohydrate intake, fat intake, and PUFA:SFA ratio between groups across pregnancy. Future potential lifestyle interventions to mediate GWG and FM gain should consider investigating diet quality and composition.

***Physical Activity:*** Physical activity may influence body composition during pregnancy and should be considered in lifestyle interventions for mediating GWG and changes in FM. Exercise is effective at mediating change in FM as women who continue their preconception exercise regimes have been shown to have a lower sum of SF in late pregnancy when compared to women who stopped exercising during pregnancy (86). Among the women enrolled in BHIP, there was a lack of adherence to the walking intervention of 10,000 steps a day. This goal was not achieved in 91% of participants in mid pregnancy and 92% at the end of pregnancy, as measured by accelerometry. Since most women in the intervention group did not adhere to the exercise component of the intervention, and that energy expenditure was similar to the control group, the similar change in FM between groups is not surprising. Future studies should evaluate the effect of relatively moderate to high intensity exercise on change in adiposity in pregnancy since in non-pregnant populations, evidence suggests high intensity exercise promotes greater lipid oxidation (87–91).

***Pre-pregnancy BMI:*** Entering pregnancy at a higher pBMI may influence the amount of fat gained during pregnancy, however, evidence to date has been inconclusive. In our study women in the overweight/obese pBMI category experienced a smaller

change in FM across pregnancy in comparison to normal weight women, regardless of how adiposity was measured (Appendix 2). This was also observed in a previous study that measured fat mass by using both hydrodensitometry and deuterium dilution (38). However, these results are not consistent as another study found that while lean weight women experienced a greater increase in %BF (measured by hydrodensitometry) across pregnancy in comparison to obese women, the total absolute amounts of FM gained were similar (92). It should be noted that while both studies use hydrodensitometry, the equations used to convert hydrodensitometry measurements to %BF or FM differed between studies, likely explaining the differences in results. A few studies have also reported a positive relationship between pBMI and amount of fat gained (39,93). Given the inconsistency between studies, there is interest to evaluate the mechanisms behind the relationship between adiposity status on entering pregnancy and changes in adiposity during pregnancy.

## 5.2 Cardiometabolic and adverse outcomes in relation to adiposity

### 5.2.1 Cardiometabolic outcomes

While cardiometabolic adaptations are expected across pregnancy, we were able to show how these related specifically to changes in adiposity. The association between accumulated fat and an array of cardiometabolic biomarkers provide insight on how adiposity can influence cardiometabolic adaptations that occur during pregnancy. This is of clinical significance as the effect of adiposity on expected cardiometabolic adaptations may impact risk of cardiometabolic dysfunction (48,72,94,95).

Our observations of the positive relationship between change in adiposity and both insulin and leptin are supported by previous research. Changes in insulin sensitivity are partly associated with maternal FM, however, the molecular mechanism has yet to be elucidated (94–96). One study in particular demonstrated that from preconception to early pregnancy, changes in FM were inversely associated with changes in relative insulin sensitivity (56). In regards to leptin, concentrations of leptin in pregnancy are positively correlated with FM (50,72,97,98). Our results demonstrate that both insulin and leptin are positively associated with pBMI as well, which is consistent with current evidence (50,96). Insulin also has an important role in fat accumulation, and thus may play a role in understanding the associations between lipid profile and change in adiposity (97,99).

The impact of adiposity on lipid profile is complex as across pregnancy there are profound changes in lipid metabolism which respond differently to different pregravid adiposity and obesity states. As demonstrated in the BHIP study and others (43), overweight and obese women enter pregnancy with a more atherogenic profile when compared to normal weight women, however, trajectories of lipid biomarkers differ. Overweight and obese women also have a smaller change in some lipid biomarkers compared to normal weight women, irrespective of GWG (45–47). In our study, overweight and obese women had higher mean triglycerides and lower HDL during pregnancy, however experienced a smaller change in total cholesterol and LDL across pregnancy compared to normal weight women. Thus, the atherogenic profile associated with greater pBMI responds differently to metabolic adaptations in pregnancy. Further, our findings suggest that greater fat accumulation during pregnancy is associated with

lower triglyceride concentrations and higher HDL concentrations. This demonstrates the complex relationship between adiposity and lipid profile as both pre-gravid adiposity and changes in fat mass during pregnancy influence maternal lipid profile.

The relationship between adiposity and SBP is not fully elucidated, as hemodynamic adaptations that occur in pregnancy have an opposing effect to obesity. The impact of obesity and adiposity on the pressure-natriuresis relationship can be a result of elevated insulin and leptin levels associated with obesity, among other factors (100,101). During pregnancy however, the development of the uteroplacental vascular system and adaptations to the renin-angiotensin-aldosterone system in mid pregnancy results in a drop in blood pressure, which then steadily increases until term (102). The negative association between change in adiposity and SBP may illustrate how greater adiposity change can influence and alter expected hemodynamic adaptations during pregnancy. A recent retrospective analysis reported that gestational hypertension was associated with higher FM in mid pregnancy while preeclampsia was associated with lower FM in early pregnancy (54). The inconsistent associations between hypertensive disorders and FM makes evident the complex relationship between hemodynamic adaptations and adiposity.

The associations found between cardiometabolic biomarkers and changes in adiposity demonstrate how adiposity can result in perturbations to expected cardiometabolic adaptations. As confirmed in our study, pBMI was associated with cardiometabolic biomarker concentrations during pregnancy, as well as change in cardiometabolic biomarker concentrations across pregnancy. Another influence on cardiometabolic status in pregnancy is diet (103). But in our study, diet was unlikely a

mitigating factor in cardiometabolic status since most dietary measures were not associated with pBMI. Women at a higher pBMI did spend less time doing moderate to vigorous physical activity. This may explain the differences in CM profile with increasing pBMI as physical activity influences cardiometabolic biomarker concentrations, however our analysis is not conclusive (90,91,104). Overall, our findings demonstrate that both pre-gravid obesity and amount of FM gained across pregnancy can result in alterations to expected cardiometabolic adaptations.

### 5.2.2 Adverse outcomes

In our analysis, change in adiposity across pregnancy had no significant effect on risk of operative vaginal delivery or caesarean section. However, the validity of these findings was limited as the sample size was too small to achieve high statistical power, therefore increasing risk for Type II errors. Evidence suggests that excessive adiposity in pregnancy increases risk for adverse maternal outcomes (52–54). One study in particular found women diagnosed with gestational hypertension had lower TBW and greater %BF at all stages of pregnancy in comparison to women who did not have gestational hypertension (54). However, it is not well understood whether change in adiposity across pregnancy impacts risk of adverse outcomes. In our study, we were unable to evaluate the association between change in adiposity and both GDM and preeclampsia due to a limited number of cases and due to missing data. Thus, it is unclear whether the effect change in adiposity has on cardiometabolic adaptations in pregnancy would result in increased risk for adverse pregnancy outcomes.

### 5.2.3 Comparing strength of association between adiposity measures

Associations between cardiometabolic biomarkers and change in adiposity across pregnancy differed between adiposity measurement tools. The associations between cardiometabolic biomarkers and changes in adiposity were consistent between sum of SFT and %BF by SFT. However, we found difference significant associations between cardiometabolic biomarkers and %BF by BIA. The varied associations between measures may be due to differences inherent to the measurement tools.

The variation in metabolic activity of different fat depots may explain the differences in findings between measurement tools. Visceral fat is more metabolically active, with greater vasculature, lipolytic activity, and glucose uptake, when compared to subcutaneous fat in non-pregnant populations (105,106). During pregnancy, there is greater deposition of visceral fat tissue in comparison to subcutaneous in the abdomen (43,44). However, deposition of abdominal visceral fat tissue varies with pre-gravid obesity, with a lower change in visceral fat tissue with increasing pBMI (43). Such changes in visceral abdominal tissue are captured in BIA measurements and may explain why BIA had different associations with cardiometabolic markers compared to SFT derived measures. Interestingly, visceral adipose tissue is strongly associated with insulin resistance in non-pregnant populations, however, we did not find any associations between BIA and insulin resistance (43,52,105,106). This may indicate that insulin resistance characteristic of pregnancy is not exclusively the result of increased visceral adipose tissue.

Limitations of each adiposity measure may also explain the difference between measures. Thus, to elucidate the mechanisms behind these findings, there is a need for more robust measures of adiposity in pregnancy. As well, determining the metabolic mechanisms may provide an improved understanding of the relationship between weight gain in pregnancy and adverse cardiometabolic outcomes.

## 5.3 New insights into measures of adiposity in pregnancy

### 5.3.1 Limitations of measurement tools

Our study contributed new insights into the congruence of readily available non-invasive measures of adiposity in pregnant women. In our comparative analysis, adiposity measures at different time points varied between measurement tools. Such discrepancies between tools may be attributed to estimation bias thus impacting the validity of measurements at different pregnancy stages. The differences between measures may also be partly attributed to the different body compartments evaluated by each measure. Across pregnancy, fat accumulates predominately in the trunk and thighs, with no significant change in subcutaneous fat in the upper arms (42). As well, there is greater utilization of fat stores in the triceps and thighs in late pregnancy in comparison to the scapular area (107). In our study, BIA measurements were taken in the legs and lower part of the abdomen, while skinfolds were mainly taken in the shoulder and arm regions, with one measurement in the abdominal area. Further, BIA measures both subcutaneous and visceral adipose tissue, while skinfolds measure only subcutaneous fat. Thus, BIA evaluates adiposity in a compartment with greater change in adiposity when compared to

body compartments evaluated by skinfolds, likely resulting in greater average %BF estimates.

The dynamic nature of pregnancy can impact adiposity tools, resulting in differences between measures at varying stages of pregnancy. Hydration increases as pregnancy progresses, potentially impacting the precision of measures that assume a constant hydration (40,108,109). Skinfold measurements are partly impacted by changes in hydration as increased hydration of connective tissues impacts the compressibility of skinfolds (40). Some equations to estimate %BF from sum of skinfolds adjust for fluid shifts, however they are specific to certain weeks of gestation (110,111). Fluid shifts across pregnancy impact BIA as it estimates %BF by determining TBW with the assumption that there is a constant ratio of intracellular to extracellular water, which does not hold in a pregnant population (108,112,113). As a result, in later stages of pregnancy, BIA underestimates TBW resulting in an overestimation of fat (41). Currently there are no equations to convert impedance measurements to %BF that are specific to a pregnant population. The impact of changes in hydration across pregnancy likely influence BIA and SFT differently, resulting in the increasing bias as pregnancy progresses. The strong agreement between the two measures plus DXA in the postpartum support this as the fetus is a significant contributor to the change in FFM across pregnancy (114). While fluid shifts across pregnancy have unique effects of BIA and skinfold measurements, it is not possible to conclude which measure was more accurate without comparing to a gold-standard method.

In the BHIP trial, we were able to assess agreement between BIA and SFT with DXA at 6 months postpartum. We observed strong agreement between DXA and BIA, as well as DXA and SFT albeit comparatively weaker. This indicates that postpartum, %BF by BIA or by SFT are valid estimates of adiposity, however BIA is preferred.

Obesity in pregnancy is also a source of estimation bias for both BIA and SFT, impacting the validity of results. Bioelectrical impedance analysis assumes the body acts as a cylindrical conductor with homogenous composition, however this assumption doesn't hold with increasing obesity due to the elevated abdominal adiposity (115). Further, both %BF estimated by BIA or SFT underestimate %BF at greater adiposity in non pregnant populations (115–117). However, the influence of obesity on estimation of %BF may differ as there was a greater difference in %BF estimated by BIA and SFT with increasing adiposity among women enrolled in BHIP. Obesity may also result in variation in fluid shifts as body water distribution is different in obese individuals when compared to leaner people, influencing both BIA and SFT (115). The estimation bias resulting from pre-gravid obesity is then important to consider when using either measure in a sample with diverse pBMI.

Skinfold measurements are subject to observer error due to limitations in conducting measurements. Unlike BIA, to obtain precise skinfold measurements trained technicians are required (40). Previous studies have reported significant observer error when measuring skinfolds, with noticeable variation with displacement of site measurement (118,119). Fortunately, in the BHIP trial, an inter-observer evaluation for SFT measures did not find significant differences in measures between observers (Appendix 4). Another

source of error is due to the difficulty of obtaining measurements at later stages of pregnancy as a result of the growing fetus impacting ability to obtain measurements in the trunk area (40).

This comparative analysis demonstrates the limitations of currently available methods in evaluating adiposity in a pregnant population. The measurement methods that we employed to assess adiposity are not equivalent, most notably in late pregnancy, due to differences between tools in underlying assumptions and areas of body fat measured. While there are other methods to evaluate adiposity, such as air displacement plethysmography, many of these other measurement tools can be costly, take more time to conduct, and are not practical in a clinical practice setting. This poses a concern in evaluating adiposity in field studies or in office practices. Further, many of these methods have methodological limitations both similar to and different from BIA and SFT, and therefore concerns prevail across methods regarding accuracy and validity. Of the available methods, most are not validated in a pregnant population, are influenced by fluid shifts across pregnancy, and cannot separate fetal and maternal contributions to body composition (40,41). These limitations are a source of variability that may explain the inconsistency in our results when comparing measurement tools.

### 5.3.2 Clinical evaluation of adiposity

Pre-pregnancy BMI is often used as a screening tool to evaluate adiposity in early pregnancy. However, pBMI has limitations as a surrogate measure of adiposity as was clearly demonstrated in our study in which pBMI discriminated fewer women as overweight/obese when compared to both BIA and SFT measures in early pregnancy. The

problem with use of pBMI as a reflection of adiposity status is that it does not reflect the distribution of FM to FFM (120). As well, pBMI doesn't consider factors that influence body composition, such as age and ethnicity (120,121). In non-pregnant populations, BMI was found to be less sensitive in discriminating between normal and overweight/obese when compared to direct adiposity measures (122), which was similar to our study.

The comparatively low sensitivity of pBMI to direct measures of adiposity is a clinical concern as women who may be at risk for adiposity related adverse outcomes are not being identified. In non-pregnant populations, normal weight individuals with obesity defined level of %BF have higher cardiometabolic risk factors when compared to normal weight individuals with normal weight defined level of %BF (123). Maternal adiposity is associated with altered cardiometabolic profile which may be reflected in increased risk for cardiometabolic dysfunction during pregnancy (48,72,94,95). It may then be preferable to use a direct measure of adiposity to screen for at-risk pregnancies instead of pBMI.

As a screening for adiposity-related adverse outcomes in early pregnancy, sum of SF or BIA may be more preferable to %BF estimated by SFT. Current equations to estimate %BF from SFT are specific to gestational stages, none of which are appropriate in early pregnancy (40). As well, most equations are derived from validation studies that do not include many obese women, and thus may not be appropriate for them (41). In this study, %BF estimated by SFT did not increase across pregnancy, while our measures of sum SFT and %BF by BIA both increased significantly from early to late pregnancy, which is inconsistent with established literature (36,38,76). This brings to question the suitability

of the equations used, even though both SFT measures had similar associations with cardiometabolic biomarkers. Finally, all limitations for measuring sum of SF are also limitations for %BF estimated by SFT. Although we cannot conclude which measure is most accurate without a gold standard method, %BF estimated by SFT has multiple methodological concerns that make it inappropriate as a screening tool in early pregnancy.

### 5.4 Strengths and limitations

Our study has several strengths. A robust evaluation of cardiometabolic profile was conducted in both early and late pregnancy, measuring biomarkers for glucose metabolism, lipid metabolism, adipocyte metabolism, and inflammation. This allowed for an objective analysis of the overall metabolic profile in pregnancy. An extensive number of quantitative and qualitative measures provided for a comprehensive evaluation of potential confounding variables. For example, a three-day diet record was used rather than an FFQ, which can be subject to recall bias. Also, physical activity was quantitatively measured using accelerometry. Dietary and physical activity measures were assessed across pregnancy, providing a thorough longitudinal analysis of lifestyle behaviours. Another strength to this study was the inclusion of multiple quantitative adiposity measurement tools in pregnancy and the post partum, as many studies evaluate obesity using pBMI only. The primarily European study sample was a strength as it minimized racial variability in the study. This is beneficial as evidence exists of racial differences in body composition and cardiometabolic health (105,121,124–126). The diverse distribution of BMI categories among the study sample was a benefit as it

provides a more comprehensive evaluation of how body composition, lifestyle factors, and cardiometabolic profile are associated as pBMI influences adiposity change and cardiometabolic biomarkers (38,39,45,93,127).

Our study also has some limitations that should be considered. Most participants were of European descent, well-educated, and had a high household income, limiting the generalizability of our findings. Our participants being mostly highly educated and wealthy may be because recruitment occurred predominately in midwifery clinics (59). In Ontario, women who visit midwifery clinics are predominately highly educated, are of a medium income bracket or higher, and are married (128). Also, there were few cases of women experiencing adverse outcomes. As a result, we excluded analyses evaluating associations between change in adiposity with risk of gestational diabetes or preeclampsia. As well, the analysis evaluating associations between change in adiposity and both caesarean section and operative vaginal delivery had low statistical power. The low risk in adverse outcomes among BHIP participants may be attributed to the high socioeconomic status of women enrolled. Previous studies have shown a reduced risk in adverse outcomes with higher socioeconomic status (129,130). As well, evidence suggests racial disparities regarding risk of adverse outcomes (131).

A limitation regarding adiposity measurement tools used in this study was that there was no gold standard method to compare measures to during pregnancy. We were able to conduct a comparison with DXA and observed good agreement in quantitation of %BF between DXA, SF and BIA; however, this was at 6 months postpartum and thus cannot determine the validity of measures conducted during pregnancy. As well, the equations

used to estimate %BF from both BIA and SFT have not been validated in a pregnant population. This limitation was a result of the limited available adiposity tools that are appropriate in a pregnant population.

We could not determine causal relationships as the analyses elucidating relationships between change in adiposity with cardiometabolic biomarker concentrations and risk of adverse outcomes were an exploratory observational analysis. Further, the limitations regarding adiposity tools impacts this analysis as the estimated change in adiposity cannot be validated. As well, the analyses would have benefitted from preconception cardiometabolic measurement as baseline measures as metabolic adaptations occur early in pregnancy and influence early placenta function (36,132,133).

### 5.5 Contributions to clinical practice and future directions

This secondary analysis of the BHIP randomized controlled trial (RCT) provides a robust evaluation of the impact of a nutrition and exercise intervention on changes in adiposity across pregnancy. Most previous studies (25–29) that explored the impact of lifestyle interventions in pregnancy focused only on gestational weight gain, but there are studies exploring the impact of lifestyle interventions on adiposity. The results of one study demonstrated that continuing regular physical activity behaviours from preconception into pregnancy resulted in significantly lower SFT at 23, 31, and 37 weeks gestation when compared to women who stopped their regular exercises (86). However, this study was not a randomized intervention study, impacting the quality of the results. In a recent intervention study in pregnant women in Sweden, dietary counselling advising women to consume 3 meals of fish per week (N = 35) did not result in significant

differences in FM or FFM compared to standard care (134). This study has limited generalizability due to the small sample size and the exclusion of underweight, overweight, and obese women. Further, dietary intake was estimated using food frequency questionnaire (FFQ) and serving size estimates, which is subject to recall bias. Another recent trial assessed the impact of a dietary and lifestyle intervention on adiposity and found no intervention effect on SFT in late pregnancy (135). The intervention used in the study was lifestyle advising 2 weeks after randomization, with biweekly advice reinforcement starting at 22 weeks gestation. The applicability of these results is limited as the study included only overweight and obese participants. As well, the authors do not have objective measures of dietary intake and physical activity and are therefore unable to assess if the lifestyle intervention resulted in significant changes between the intervention and control groups. This would be valuable to consider given they found no intervention effect. Our study addresses many of the limitations present in these previous studies, with a larger sample size, diverse pBMI, and quantitative measures for diet and physical activity to provide an objective assessment of the intervention. Future studies should explore other lifestyle interventions to add to this research.

The study provides an overview of cardiometabolic profiles in both early and late pregnancy from a large cohort of Canadian women. Population based data on health practises of Canadian women are not available as currently the Canadian Health Measures Survey do not report on pregnant women. This study also adds to our understanding of the relationship between pre-gravid obesity and cardiometabolic profile, as well as

providing new insight on how changes in adiposity during pregnancy are associated with cardiometabolic profile. We found that dietary intake did not differ between weight categories, indicating that differences in cardiometabolic profile between weight categories were not a result of diet. Time spent doing moderate to vigorous physical activity did differ between weight categories and thus may have had a role in the differences found. However, despite adjusting for both dietary and physical activity measures, associations between changes in adiposity and cardiometabolic biomarker concentrations remained. These findings then support the need to address potential cardiometabolic health concerns in preconception.

The findings from this analysis demonstrate the limitation of available measures to quantify adiposity in pregnancy and the need for validated measures. Pre-pregnancy BMI is often used as a screening tool for adiposity, however pBMI is less sensitive compared to %BF estimates by BIA and SFT. Given this limitation, non-invasive adiposity measurement tools may be preferred to screen for potential at-risk pregnancies. However, current methods are subject to varying limitations impacting validity of measures. Further, our findings demonstrate differences between measurement tools regarding associations between changes in adiposity and cardiometabolic biomarkers. Thus, the lack of valid adiposity measures poses a concern as the measurement tool used is a source of significant variation. Future research efforts should explore validating body composition techniques for use in pregnancy that are feasible in large-scale cohort studies, as well as clinical settings.

# CHAPTER 6

# APPENDICES

# CHAPTER 6 - APPENDICES

## 6.1 APPENDIX 1 - ASSAYS USED TO MEASURE CARDIOMETABOLIC BIOMARKERS

For fasting plasma glucose, a 500µL aliquot of plasma collected from the sodium fluoride/$Na_2$ EDTA tube was required for analysis. Plasma glucose concentration was estimated using a hexokinase photometric assay. The assay coefficient of variation was ≤5%, and the system was calibrated approximately every 30 days with a calibration curve ranging from 0.28 to 44.40 mmol/L. Samples were tested directly unless values exceeded 44 mmol/L where instead samples were diluted 1:5 (Abbott Architect *ci*4100, Abbott Park, IL).

For lipid profile concentrations, a 500µL serum aliquot from the SST™ serum separation tube was analyzed. An enzymatic assay was used to measure total cholesterol. The assay coefficient of variation was ≤3% and the system is calibrated approximately every 30 days with a calibration curve ranging from 0 mmol/L to 18.26 mmol/L. Samples were tested undiluted, with an automatic 1:4 dilution being performed if sample values exceed 18.26 mmol/L (Abbott Architect *ci*4100, Abbott Park, IL). To measure HDL concentrations, an accelerator selective detergent was used. This assay has a coefficient of variation of ≤4% and was calibrated approximately every 28 days with a calibration curve ranging from 0 mmol/L to 4.66 mmol/L. Samples are tested undiluted unless values exceed 4.66 mmol/L, in which the sample is diluted to no longer exceed 4.66 mmol/L (Abbott Architect *ci*4100, Abbott Park, IL). Triglyceride values were measured using glycerol phosphate oxidase assay. Glycerol phosphate oxidase assay has a coefficient of variation of ≤5% and the assay was calibrated approximately every 41 days with a

calibration curve ranging from 0 mmol/L to 16.05 mmol/L. Samples were tested undiluted, however, values that exceed 16.05 mmol/L has a 1:4 dilution performed and are re-tested (Abbott Architect *ci*4100, Abbott Park, IL). Low density lipoprotein was calculated based on measured total cholesterol, HDL, and triglyceride values (Abbott Architect *ci*4100, Abbott Park, IL).

Fasting serum leptin and insulin were measured in duplicate using a Luminex® human premixed multi-analyte ELISA (R&D Systems, Minneapolis MN). Plasma samples were run in triplicate for each plate for quality control. For both insulin and leptin, a sample volume of 50µL was used with a 1:2 dilution factor. Before each use of the Bio-Rad Bio Plex® 200 system, it was calibrated. Validation of the Bio-Rad Bio Plex® system was conducted monthly. The intra- and interassay coefficients of variation were 5.7% and 15.6% for leptin, and 4.9% and 20.1% for insulin, respectively.

Fasting serum adiponectin and CRP were measured by Luminex® premixed multi-analyte enzyme-linked immunosorbent assay (R&D Systems, Minneapolis MN) utilizing the same protocol for insulin and leptin, except for a 1:500 dilution factor being used. Intra- and interassay coefficients of variation were 8.0% and 11.8% for adiponectin, and 6.1% and 11.5% for CRP, respectively.

## 6.2 APPENDIX 2 - ADIPOSITY TRENDS STRATIFIED BY WEIGHT CATEGORIES

**Figure 6.2.1** Sum of skinfolds vs. gestational age stratified by pBMI. Normal weight: r = 0.2262, p = <0.001; Overweight/obese: r = 0.0473, p = 0.46, *n* = 191

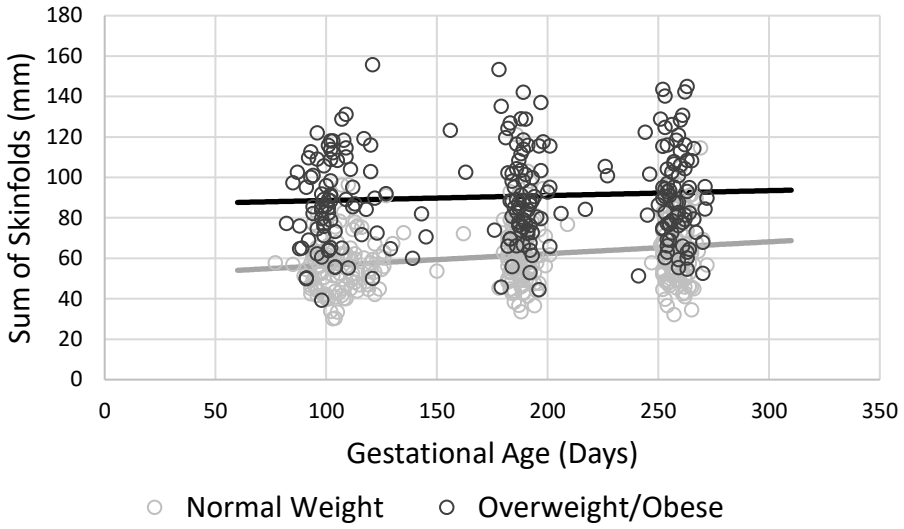



**Figure 6.2.2** Percent BF by SFT vs. gestational age stratified by pBMI. Normal weight: r = -0.0457, p = 0.41; Overweight/obese: r = -0.1794, p = 0.005, *n* = 191

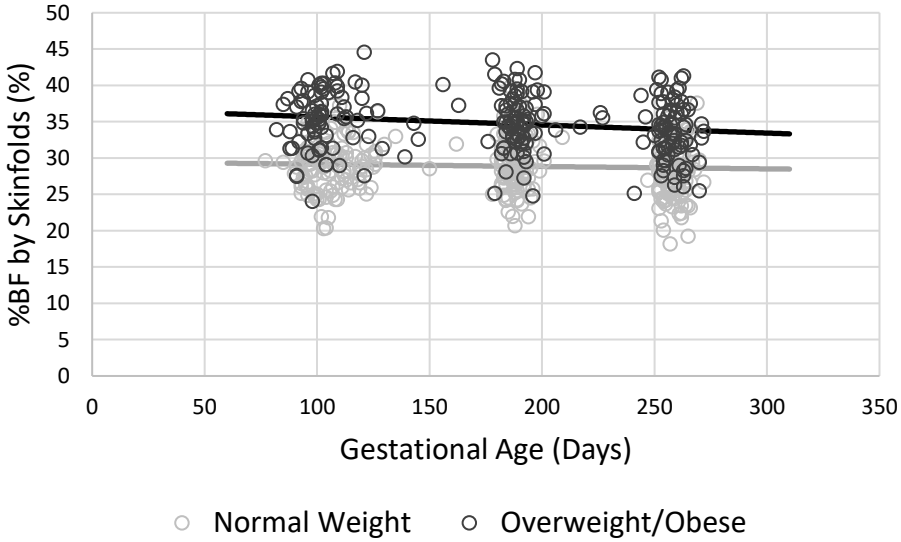


**Figure 6.2.3** Percent BF by BIA vs. gestational age stratified by pBMI. Normal weight: r = 0.4027, p = <0.001; Overweight/obese: r = 0.2364, p = <0.001, *n* = 182

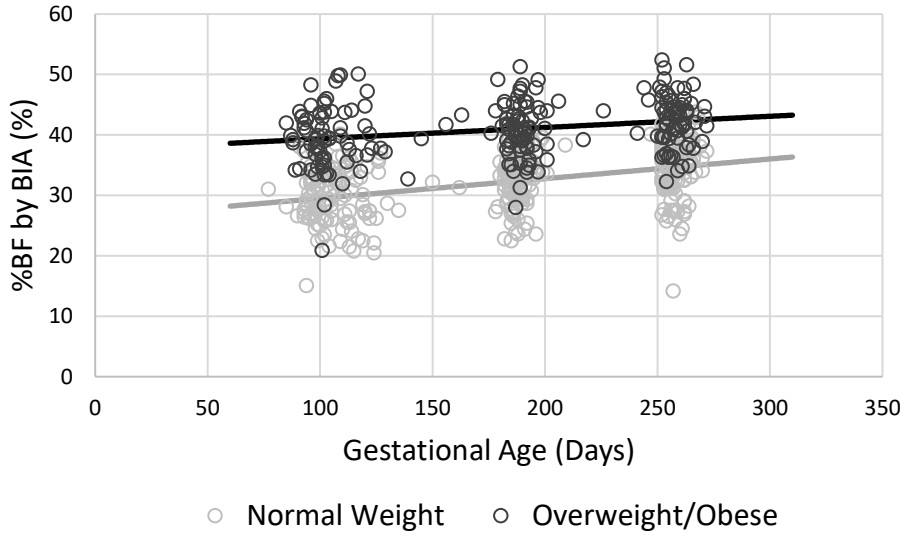



**Figure 6.2.4** Percent BF by SFT vs. gestational age stratified by %BF weight categories. Normal weight: r = -0.0384, p = 0.51; Overweight/obese: r = -0.2130, p = <0.001, *n* = 191

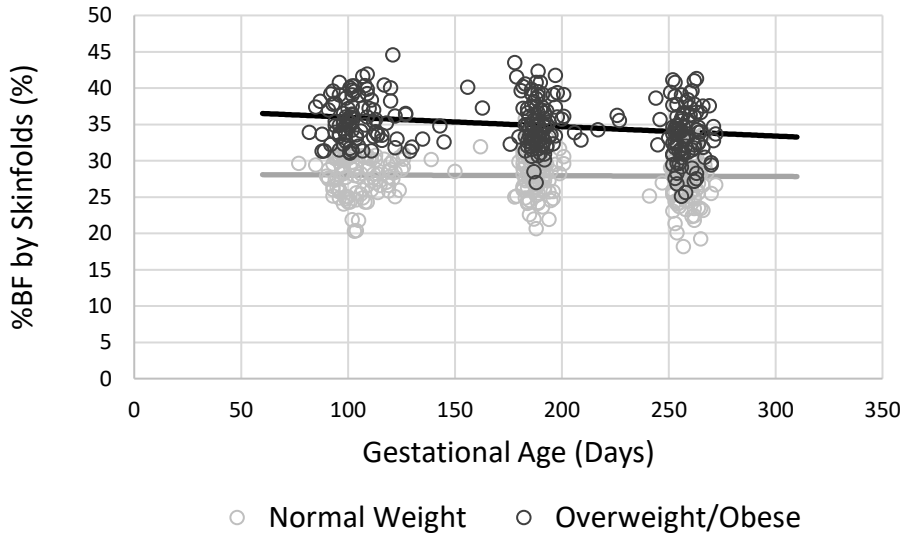


**Figure 6.2.5** Percent BF by BIA vs. gestational age stratified by %BF weight categories. Normal weight: r = 0.5469, p = <0.001; Overweight/obese: r = 0.2813, p = <0.001, *n* = 182

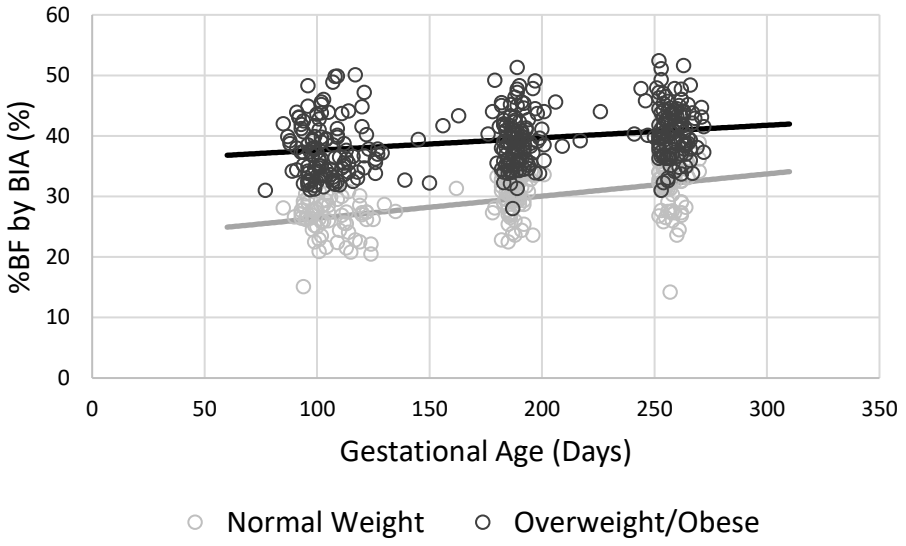



**Table 6.2.1** Mean change in adiposity from early to late pregnancy stratified by pBMI.

| **Outcome**[1] | **Normal Weight** | **Overweight/Obese** | ***p*** |
|---|---|---|---|
| Sum of SFT (mm) | 8.68 ± 12.22 | 4.09 ± 15.5 | 0.054 |
| %BF by SFT (%) | -0.60 ± 2.79 | -1.74 ± 2.56 | 0.014 |
| %BF by BIA (%) | 4.84 ± 3.66 | 3.00 ± 3.00 | 0.001 |

[1]Adjusted for age, ethnicity, parity, study site, and study arm.

## 6.3 Appendix 3 – Demographics comparison between analyzed and excluded participants

**Table 6.3.1** Comparison of baseline demographic characteristics of study participants included in the treatment analysis and participants that were excluded.

| Maternal characteristics | Included Participants N = 192 | Excluded Participants N = 49 | *p*[1] |
|---|---|---|---|
| Gestational age at enrollment (wk) median (Q1, Q3) | 14.7 (13.7, 16.0) | 14.9 (13.6, 16.2) | 0.016 |
| Maternal age (yr) median (Q1, Q3) | 31 (29, 34) | 31 (28, 33) | 0.362 |
| Pre-pregnancy BMI ($kg/m^2$) median (Q1, Q3) | 24.3 (21.9, 27.2) | 26.9 (22.6, 30.2) | 0.016 |
| Underweight N (%) | 3 (1.6) | 1 (2.0) | 0.029 |
| Normal weight N (%) | 109 (56.8) | 17 (36.7) | |
| Overweight N (%) | 51 (26.6) | 18 (36.7) | |
| Obese N (%) | 29 (15.1) | 3 (6.1) | |
| Ethnicity, N (%) | | | 0.302 |
| European descent | 173 (90.1) | 39 (79.6) | |
| Mixed | 9 (4.7) | 4 (8.2) | |
| Other | 10 (5.2) | 4 (8.2) | |
| Unknown/prefer not to answer | 0 (0.0) | 2 (4.1) | |
| Household income, N (%) | | | 0.040 |
| Household income ≥ $75000 | 141 (73.4) | 28 (57.1) | |
| Unknown/prefer not to answer | 8 (4.2) | 3 (6.1) | |
| Highest level of education, N (%) | | | 1.000 |
| Tertiary | 178 (92.7) | 45 (91.8) | |
| Unknown/prefer not to answer | 5 (2.6) | 2 (4.1) | |
| Parity, N (%) | | | 0.621 |
| 0 | 94 (49.0) | 20 (40.1) | |
| 1+ | 97 (50.5) | 25 (51.0) | |
| Unknown/prefer not to answer | 1 (0.5) | 4 (8.2) | |

[1]To assess differences Mann Whitney U test was used for non-normal continuous data, Fisher's exact test was used for categorical data

## 6.4 APPENDIX 4 – BHIP INTER-OBSERVER EVALUATION OF 4-SITE SKINFOLD THICKNESS

**Table 6.4.1** T-test comparison of triplicate 4-site SFT sums between all observers in study.

| | ***P*** | | | |
|---|---|---|---|---|
| | Observer A | Observer B | Observer C | Observer D |
| Observer A | - | 0.878 | 0.769 | 0.784 |
| Observer B | - | - | 0.664 | 0.705 |
| Observer C | - | - | - | 0.965 |
| Observer D | - | - | - | - |

## REFERENCES

1. Kowal C, Kuk J, Tamim H. Characteristics of weight gain in pregnancy among canadian women. Matern Child Health J. 2012;16(3):668–76.

2. Lowell H, Miller DC. Weight gain during pregnancy: adherence to Health Canada's guidelines. Health Rep. 2010;21(2):31–6.

3. Institute of Medicine. Weight gain during pregnancy: reexamining the guidelines. Institute of Medicine of the National Academies. 2009.

4. Dzakpasu S, Fahey J, Kirby RS, Tough SC, Chalmers B, Heaman MI, et al. Contribution of prepregnancy body mass index and gestational weight gain to adverse neonatal outcomes: Population attributable fractions for Canada. BMC Pregnancy Childbirth. 2015;15(1):1–12.

5. Begum F, Colman I, McCargar LJ, Bell RC, on behalf of the Alberta Pregnancy Outcomes. Gestational Weight Gain and Early Postpartum Weight Retention in a Prospective Cohort of Alberta Women. J Obstet Gynaecol Canada [Internet]. 2012;34(7):637–47. Available from: http://dx.doi.org/10.1016/S1701-2163(16)35316-6

6. Li A, Teo KK, Morrison KM, McDonald SD, Atkinson SA, Anand SS, et al. A genetic link between prepregnancy body mass index, postpartum weight retention, and offspring weight in early childhood. Obesity. 2017;25(1):236–43.

7. Yu CKH, Teoh TG, Robinson S. Obesity in pregnancy. BJOG An Int J Obstet

Gynaecol. 2006;113(10):1117–25.

8. Ren M, Li H, Cai W, Niu X, Ji W, Zhang Z, et al. Excessive gestational weight gain in accordance with the IOM criteria and the risk of hypertensive disorders of pregnancy: A meta-analysis. BMC Pregnancy Childbirth. 2018;18(1):1–9.

9. Catalano PM, Ehrenberg HM. The short- and long-term implications of maternal obesity on the mother and her offspring. BJOG An Int J Obstet Gynaecol. 2006;113(10):1126–33.

10. Catalano PM, McIntyre HD, Cruickshank JK, McCance DR, Dyer AR, Metzger BE, et al. The hyperglycemia and adverse pregnancy outcome study: Associations of GDM and obesity with pregnancy outcomes. Diabetes Care. 2012;35(4):780–6.

11. Athukorala C, Rumbold AR, Willson KJ, Crowther CA. The risk of adverse pregnancy outcomes in women who are overweight or obese. BMC Pregnancy Childbirth. 2010;10.

12. Brunner S, Stecher L, Ziebarth S, Nehring I, Rifas-Shiman SL, Sommer C, et al. Excessive gestational weight gain prior to glucose screening and the risk of gestational diabetes: a meta-analysis. Diabetologia. 2015;58(10):2229–37.

13. Dzakpasu S, Fahey J, Kirby RS, Tough SC, Chalmers B, Heaman MI, et al. Contribution of prepregnancy body mass index and gestational weight gain to caesarean birth in Canada. BMC Pregnancy Childbirth [Internet]. 2014;14(1):1–8. Available from: BMC Pregnancy and Childbirth

14. Durst JK, Sutton ALM, Cliver SP, Tita AT, Biggio JR. Impact of Gestational Weight Gain on Perinatal Outcomes in Obese Women. Am J Perinatol. 2016;33(9):849–55.

15. Garovic VD, August P. Preeclampsia and the future risk of hypertension: The pregnant evidence. Curr Hypertens Rep. 2013;15(2):114–21.

16. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet [Internet]. 2009;373(9677):1773–9. Available from: http://dx.doi.org/10.1016/S0140-6736(09)60731-5

17. Gaudet L, Ferraro ZM, Wen SW, Walker M. Maternal obesity and occurrence of fetal macrosomia: A systematic review and meta-analysis. Biomed Res Int. 2014;2014.

18. Eriksson JG, Sandboge S, Salonen MK, Kajantie E, Osmond C. Long-term consequences of maternal overweight in pregnancy on offspring later health: Findings from the Helsinki Birth Cohort Study. Ann Med. 2014;46(6):434–8.

19. Oken E, Rifas-Shiman SL, Field AE, Frazier AL, Gillman MW. Maternal Gestational Weight Gain and Offspring Weight in Adolescence. Obstet Gynecol. 2008;112(5):999–1006.

20. Gregg EW, Chen H, Wagenknecht LE, Clark JM, Delahanty LM, Bantle J, et al. Association of an intensive lifestyle intervention with remission of type 2 diabetes.

JAMA - J Am Med Assoc. 2012;308(23):2489–96.

21. BA S, I C, JC S, WPT J. Diet, nutrition and the prevention of excess weight gain and obesity. Public Health Nutr. 2004;7(1a):123–46.

22. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279–90.

23. Wadden TA, Webb VL, Moran CH, Bailer BA. Lifestyle modification for obesity: New developments in diet, physical activity, and behavior therapy. Circulation. 2012;125(9):1157–70.

24. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in Diet and Lifestyle and Long-term Weight Gain in Women and Men. N Engl J Med. 2011;364(25):2392–404.

25. Peaceman AM, Clifton RG, Phelan S, Gallagher D, Evans M, Redman LM, et al. Lifestyle Interventions Limit Gestational Weight Gain in Women with Overweight or Obesity: LIFE-Moms Prospective Meta-Analysis. Obesity. 2018;26(9):1396–404.

26. Guelinckx I, Devlieger R, Mullie P, Vansant G. Effect of lifestyle intervention on dietary habits, physical activity, and gestational weight gain in obese pregnant women: A randomized controlled trial. Am J Clin Nutr. 2010;91(2):373–80.

27. Wilcox S, Liu J, Addy CL, Turner-McGrievy G, Burgis JT, Wingard E, et al. A

randomized controlled trial to prevent excessive gestational weight gain and promote postpartum weight loss in overweight and obese women: Health In Pregnancy and Postpartum (HIPP). Contemp Clin Trials [Internet]. 2018;66(November 2017):51–63. Available from: https://doi.org/10.1016/j.cct.2018.01.008

28. Hui AL, Back L, Ludwig S, Gardiner P, Sevenhuysen G, Dean HJ, et al. Effects of lifestyle intervention on dietary intake, physical activity level, and gestational weight gain in pregnant women with different pre-pregnancy body mass index in a randomized control trial. BMC Pregnancy Childbirth [Internet]. 2014;14(331):1–9. Available from: http://www.biomedcentral.com/content/pdf/1471-2393-14-331.pdf

29. Muktabhant B, Theresa L, Pisake L, Malinee L. Diet or exercise, or both, for preventing excessive weight gain in pregnancy. Cochrane Database Syst Rev. 2015;(6).

30. Ruiz JR, Perales M, Pelaez M, Lopez C, Lucia A, Barakat R. Supervised exercise-based intervention to prevent excessive gestational weight gain: A randomized controlled trial. Mayo Clin Proc [Internet]. 2013;88(12):1388–97. Available from: http://dx.doi.org/10.1016/j.mayocp.2013.07.020

31. Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. BMJ [Internet]. 2014;348(February):5–7. Available from: http://dx.doi.org/doi:10.1136/bmj.g1285

32. Vinter CA, Jensen DM, Ovesen P, Beck-Nielsen H, Jørgensen JS. The LiP (Lifestyle in Pregnancy) study: A randomized controlled trial of lifestyle intervention in 360 obese pregnant women. Diabetes Care. 2011;34(12):2502–7.

33. Thangaratinam S, Rogozińska E, Jolly K, Glinkowski S, Roseboom T, Tomlinson JW, et al. Effects of interventions in pregnancy on maternal weight and obstetric outcomes: Meta-analysis of randomised evidence. BMJ. 2012;344(7858):1–15.

34. Lammi-Keefe CJ, Couch SC, Kirwan JP. Handbook of Nutrition and Pregnancy. Second. Bendich A, Bales CW, editors. Handbook of Nutrition and Pregnancy. Gewerbestrasse: Springer International Publishing; 2018.

35. Larciprete G, Valensise H, Vasapollo B, Altomare F, Sorge R, Casalino B, et al. Body composition during normal pregnancy: Reference ranges. Acta Diabetol. 2003;40(SUPPL. 1):225–32.

36. Lain KY, Catalano PM. Metabolic changes in pregnancy. Clin Obstet Gynecol. 2007;50(4):938–48.

37. Scholl TO, Hediger ML, Schall JI, Khoo C-S, Fischer RL. Maternal growth for nutrients during pregnancy. Am J Clin Nutr. 1994;60(2):182–8.

38. Lederman SA, Paxton A, Heymsfield SB, Wang J, Thornton J, Pierson RN. Body fat and water changes during pregnancy in women with different body weight and weight gain. Obstet Gynecol. 1997;90(4):483–8.

39. Berggren EK, Groh-Wargo S, Presley L, Hauguel-De-Mouzon S, Catalano PM.

Maternal fat, but not lean, mass is increased among overweight/obese women with excess gestational weight gain. Am J Obstet Gynecol. 2016;214(6).

40. Widen EM, Gallagher D. Body composition changes in pregnancy: Measurement, predictors and outcomes. Eur J Clin Nutr. 2014;68(6):643–52.

41. Most J, Marlatt KL, Altazan AD, Redman LM. Advances in assessing body composition during pregnancy. Eur J Clin Nutr [Internet]. 2018;72(5):645–56. Available from: http://dx.doi.org/10.1038/s41430-018-0152-8

42. Sohlstrom A, Forsum E. Changes in adipose tissue volume and distribution during reproduction in Swedish women as assessed by magnetic resonance imaging. Am J Clin Nutr. 1995;61(2):287–95.

43. Straughen JK, Trudeau S, Misra VK. Changes in adipose tissue distribution during pregnancy in overweight and obese compared with normal weight women. Nutr Diabetes [Internet]. 2013;3(AUGUST):e84-5. Available from: http://dx.doi.org/10.1038/nutd.2013.25

44. Kinoshita T, Itoh M. Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: The ratio of visceral fat to subcutaneous fat in the abdomen. Gynecol Obstet Invest. 2006;61(2):115–8.

45. Scifres CM, Catov JM, Simhan HN. The impact of maternal obesity and gestational weight gain on early and mid-pregnancy lipid profiles. Obesity. 2014;22(3):932–8.

46. Bozkurt L, Göbl CS, Hörmayer AT, Luger A, Pacini G, Kautzky-Willer A. The impact of preconceptional obesity on trajectories of maternal lipids during gestation. Sci Rep [Internet]. 2016;6(July):1–7. Available from: http://dx.doi.org/10.1038/srep29971

47. Vahratian A, Misra VK, Trudeau S, Misra DP. Prepregnancy body mass index and gestational age-dependent changes in lipid levels during pregnancy. Obstet Gynecol. 2010;116(1):107–13.

48. Ramsay JE, Ferrell WR, Crawford L, Michael Wallace A, Greer IA, Sattar N. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. J Clin Endocrinol Metab. 2002;87(9):4231–7.

49. Mills JL, Jovanovic L, Knopp R, Aarons J, Conley M, Park E, et al. Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: The diabetes in early pregnancy study. Metabolism. 1998;47(9):1140–4.

50. Tessier DR, Ferraro ZM, Gruslin A. Role of leptin in pregnancy: Consequences of maternal obesity. Placenta [Internet]. 2013;34(3):205–11. Available from: http://dx.doi.org/10.1016/j.placenta.2012.11.035

51. Nien JK, Mazaki-Tovi S, Romero R, Erez O, Kusanovic JP, Gotsch F, et al. Plasma adiponectin concentrations in non-pregnant, normal and overweight pregnant women. J Perinat Med. 2007;35(6):522–31.

52. De Souza LR, Kogan E, Berger H, Alves JG, Lebovic G, Retnakaran R, et al. Abdominal adiposity and insulin resistance in early pregnancy. J Obstet Gynaecol Canada [Internet]. 2014;36(11):969–75. Available from: http://dx.doi.org/10.1016/S1701-2163(15)30409-6

53. Vega-Sanchez R, Barajas-Vega HA, Rozada G, Espejel-Nuñez A, Beltran-Montoya J, Vadillo-Ortega F. Association between adiposity and inflammatory markers in maternal and fetal blood in a group of Mexican pregnant women. Br J Nutr. 2010;104(12):1735–9.

54. Pisani I, Tiralongo GM, Lo Presti D, Gagliardi G, Farsetti D, Vasapollo B, et al. Correlation between maternal body composition and haemodynamic changes in pregnancy: different profiles for different hypertensive disorders. Pregnancy Hypertens [Internet]. 2017;10:131–4. Available from: https://doi.org/10.1016/j.preghy.2017.07.149

55. Mamun AA, Callaway LK, O'Callaghan MJ, Williams GM, Najman JM, Alati R, et al. Associations of maternal pre-pregnancy obesity and excess pregnancy weight gains with adverse pregnancy outcomes and length of hospital stay. BMC Pregnancy Childbirth [Internet]. 2011;11(1):62. Available from: http://www.biomedcentral.com/1471-2393/11/62

56. Catalano PM, Roman-Drago NM, Amini SB, Sims EAH. Longitudinal changes in body composition and energy balance in lean women with normal and abnormal glucose tolerance during pregnancy. Am J Obstet Gynecol. 1998;179(1):156–65.

57. Tanentsapf I, Heitmann BL, Adegboye ARA. Systematic review of clinical trials on dietary interventions to prevent excessive weight gain during pregnancy among normal weight, overweight and obese women. BMC Pregnancy Childbirth. 2011;11(81).

58. Ruifrok AE, Van Poppel MNM, Van Wely M, Rogozińska E, Khan KS, De Groot CJM, et al. Association between weight gain during pregnancy and pregnancy outcomes after dietary and lifestyle interventions: A meta-analysis. Am J Perinatol. 2014;31(5):353–64.

59. Perreault M, Atkinson SA, Mottola MF, Phillips SM, Bracken K, Hutton EK, et al. Structured diet and exercise guidance in pregnancy to improve health in women and their offspring: Study protocol for the Be Healthy in Pregnancy (BHIP) randomized controlled trial. Trials [Internet]. 2018;19(691). Available from: https://doi.org/10.1186/s13063-018-3065-x

60. Canada H. Prenatal Nutrition Guidelines for Health Professionals. Health Canada. 2010.

61. Haarbo J, Gotfredsen A, Hassager C, Christiansen C. Validation of body composition by dual energy X-ray absorptiometry (DEXA). Clin Physiol. 1991;11(4):331–41.

62. Barbosa-Silva MCG, Barros AJD. Bioelectrical impedance analysis in clinical practice: A new perspective on its use beyond body composition equations. Curr Opin Clin Nutr Metab Care. 2005;8(3):311–7.

63. Durnin JVGA, Rahaman MM. The assessment of the amount of fat in the human body from measurements of skinfold thickness. Br J Nutr. 1967;21(3):681–9.

64. van Raaij JM, Peek ME, Vermaat-Miedema SH, Schonk CM, Hautvast JG. New equations for estimating body fat mass in pregnancy from body density or total body water. Am J Clin Nutr. 1988;48(1):24–9.

65. Lin LI. A Concordance Correlation Coefficient to Evaluate Reproducibility. Biometrics. 1989;45(1):255–68.

66. Westfall PH, Troendle JF, Pennello G. Multiple McNemar Tests. Biometrics. 2010;66(4):1185–91.

67. Pencina MJ, D'Agostino Sr. RB. Evaluating Discrimination of Risk Prediction Models The C Statistic. J Am Med Assoc. 2015;314(10):1063–4.

68. Collins KH, Sharif B, Sanmartin C, Reimer RA, Herzog W, Chin R, et al. Association of body mass index (BMI) and percent body fat among BMI-defined non-obese middle-aged individuals: Insights from a population-based Canadian sample. Can J Public Heal. 2016;107(6):e520–5.

69. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Elsevier Health Sciences. 2018. 518–538 p.

70. Sonagra AD. Normal Pregnancy- A State of Insulin Resistance. J Clin Diagnostic Res. 2014;3–5.

71. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory

studies: A reference table for clinicians. Obstet Gynecol. 2009;114(6):1326–31.

72. Highman TJ, Friedman JE, Huston LP, Wong WW, Catalano PM. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. Am J Obstet Gynecol. 1998;178(5):1010–5.

73. Pitiphat W, Gillman MW, Joshipura KJ, Paige L, Douglass CW, Rich-edwards JW. Plasma C-reactice Protein in Early Pregnancy and Preterm Delivery. Am J Epidemiol. 2007;162(11):1108–13.

74. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Sheridan B, Hod M, et al. Hyperglycemia and adverse pregnancy outcome (HAPO) study: Associations with neonatal anthropometrics. Diabetes. 2009;58(2):453–9.

75. Wang M. Generalized Estimating Equations in Longitudinal Data Analysis: A Review and Recent Developments. Adv Stat. 2014;2014:1–11.

76. Kopp-Hoolihan LE, Van Loan MD, Wong WW, King JC. Fat mass deposition during pregnancy using a four-component model. J Appl Physiol. 1999;87(1):196–202.

77. Hall KD, Guo J. Obesity Energetics: Body Weight Regulation and the Effects of Diet Composition. Gastroenterology. 2017;152(7):1718–27.

78. Strasser B, Spreitzer A, Haber P. Fat loss depends on energy deficit only, independently of the method for weight loss. Ann Nutr Metab. 2007;51(5):428–32.

79. Lumey LH. Decreased birthweights in infants after maternal in utero exposure to

the Dutch famine of 1944–1945. Paediatr Perinat Epidemiol. 1992;6(2):240–53.

80. Dewey KG, McCrory MA. Effects of dieting and physical activity on pregnancy and lactation. Am J Clin Nutr. 1994;59(2 SUPPL.):446–53.

81. Carmichael SL, Shaw GM, Schaffer DM, Laurent C, Selvin S. Dieting Behaviors and Risk of Neural Tube Defects. Am J Epidemiol. 2003;158(12):1127–31.

82. Miller WC, Lindeman AK, Wallace J, Niederpruem M. Diet composition, energy intake, and exercise in relation to body fat in men and women. Am J Clin Nutr. 1990;52(3):426–30.

83. Volek JS, Sharman MJ, Gómez AL, Judelson DA, Rubin MR, Watson G, et al. Comparison of energy-restricted very low-carbohydrate and low-fat diets on weight loss and body composition in overweight men and women. Nutr Metab. 2004;1:1–13.

84. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Increased Consumption of Dairy Foods and Protein during Diet-and Exercise-Induced Weight Loss Promotes Fat Mass Loss and Lean Mass Gain in Overweight and Obese Premenopausal women. J Nutr [Internet]. 2011;141:1626–34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3159052/pdf/nut14101626.pdf

85. Phillips SM, van Loon LJC. Dietary protein for athletes: From requirements to optimum adaptation. J Sports Sci. 2011;29(SUPPL. 1).

86. Clapp JF, Little KD. Effect of recreational exercise on pregnancy weight gain and

subcutaneous fat deposition. Vol. 27, Medicine and Science in Sports and Exercise. 1995. p. 170–7.

87. De Feo P. Is high-intensity exercise better than moderate-intensity exercise for weight loss? Nutr Metab Cardiovasc Dis [Internet]. 2013;23(11):1037–42. Available from: http://dx.doi.org/10.1016/j.numecd.2013.06.002

88. Tremblay A, Simoneau JA, Bouchard C. Impact of exercise intensity on body fatness and skeletal muscle metabolism. Metabolism. 1994;43(7):814–8.

89. Boutcher SH. High-intensity intermittent exercise and fat loss. J Obes. 2011;2011.

90. Trapp EG, Chisholm DJ, Freund J, Boutcher SH. The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. Int J Obes. 2008;32(4):684–91.

91. Danielsen KK, Svendsen M, Mæhlum S, Sundgot-borgen J. Changes in Body Composition, Cardiovascular Disease Risk Factors, and Eating Behavior after an Intensive Lifestyle Intervention with High Volume of Physical Activity in Severely Obese Subjects; A Prospective Clinical Controlled Trial. J Obes. 2013;2013.

92. Ehrenberg HM, Huston-Presley L, Catalano PM. The influence of obesity and gestational diabetes mellitus on accretion and the distribution of adipose tissue in pregnancy. Am J Obstet Gynecol. 2003;189(4):944–8.

93. Butte NF, Ellis KJ, Wong WW, Hopkinson JM, O'Brian Smith E. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. Am

J Obstet Gynecol. 2003;189(5):1423–32.

94. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. Reproduction. 2010;140(3):365–71.

95. Svensson H, Wetterling L, Bosaeus M, Odén B, Odén A, Jennische E, et al. Body fat mass and the proportion of very large adipocytes in pregnant women are associated with gestational insulin resistance. Int J Obes (Lond). 2016;40(4):646–53.

96. Kampmann U, Knorr S, Fuglsang J, Ovesen P. Determinants of Maternal Insulin Resistance during Pregnancy: An Updated Overview. J Diabetes Res. 2019;2019(Figure 1).

97. Herrera E, Ortega-Senovilla H. Maternal lipid metabolism during normal pregnancy and its implications to fetal development. Clin Lipidol. 2010;5(6):899–911.

98. Van Der Wijden CL, Delemarre-Van Der Waal HA, Van Mechelen W, Van Poppel MNM. The concurrent validity between leptin, BMI and skin folds during pregnancy and the year after. Nutr Diabetes [Internet]. 2013;3(SEPTEMBER):e86-6. Available from: http://dx.doi.org/10.1038/nutd.2013.27

99. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care. 2007;30(SUPPL. 2).

100. Narkiewicz K. Obesity and hypertension - The issue is more complex than we thought. Nephrol Dial Transplant. 2006;21(2):264–7.

101. Re RN. Obesity-related hypertension. Ochsner J. 2009;9(3):133–6.

102. Grindheim G, Estensen ME, Langesaeter E, Rosseland LA, Toska K. Changes in blood pressure during healthy pregnancy: A longitudinal cohort study. J Hypertens. 2012;30(2):342–50.

103. Martin CL, Siega-Riz AM, Sotres-Alvarez D, Robinson WR, Perrin EM, Stuebe AM, et al. Maternal Dietary Patterns are Associated with Lower Levels of Cardiometabolic Markers during Pregnancy. Paediatr Perinat Epidemiol. 2016;30(3):246–55.

104. Goris AHC, Westerterp KR. Physical activity, fat intake and body fat. Physiol Behav. 2008;94(2):164–8.

105. Ibrahim MM. Subcutaneous and visceral adipose tissue: Structural and functional differences. Obes Rev. 2010;11(1):11–8.

106. Després JP. Obestiy and lipid metabolism: relevance of body fat distribution. Curr Opin Lipidol. 1991;2:5–15.

107. Sidebottom AC, Brown JE, Jacobs DR. Pregnancy-related changes in body fat. Eur J Obstet Gynecol Reprod Biol. 2001;94(2):216–23.

108. Lof M, Forsum E. Hydration of fat-free mass in healthy women with special reference to the effect of pregnancy. Am J Clin Nutr. 2004;80(4):960–5.

109. Wang ZM, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: Review and critique of a classic body-composition constant. Am J Clin Nutr. 1999;69(5):833–41.

110. Paxton A, Lederman SA, Heymsfield SB, Wang J, Thornton JC, Pierson RN. Anthropometric equations for studying body fat in pregnant women. Am J Clin Nutr. 1998;67(1):104–10.

111. J.A. H, R.W. P, B. A, K.P. H, H.N. S, Hutcheon JA, et al. A weight-gain-for-gestational-age z score chart for the assessment of maternal weight gain in pregnancy. Am J Clin Nutr [Internet]. 2013;97(5):1062–7. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=medl&NEWS=N&AN=23466397%5Cnhttp://ajcn.nutrition.org/content/97/5/1062.full.pdf+html%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed15&NEWS=N&AN=368825145

112. Kushner R, Gudivaka R, Schoeller D. Clinical characteristics influencing analysis measurements. Am J Clin Nutr [Internet]. 1996;64(suppl):423S-7S. Available from: http://ajcn.nutrition.org/content/64/3/423S.short

113. Lukaski HC, Hall CB, Siders WA. Assessment of change in hydration in women during pregnancy and postpartum with bioelectrical impedance vectors. Nutrition. 2007;23(7–8):543–50.

114. Pitkin RM. Nutritional Support in Obstetrics and Gynecology. Clin Obstet Gynecol. 1976;19(3).

115. Coppini LZ, Waitzberg DL, Campos ACL. Limitations and validation of bioelectrical impedance analysis in morbidly obese patients. Curr Opin Clin Nutr Metab Care. 2005;8(3):329–32.

116. Wattanapenpaiboon N, Lukito W, Strauss BJG, Hsu-Hage BH, Wahlqvist ML, Stroud DB. Agreement of skinfold measurement and bioelectrical impedance analysis (BIA) methods with dual energy X-ray absorptiometry (DEXA) in estimating total body fat in Anglo-celtic Australians. Int J Obes Relat Metab Disord. 1998;22(9):9854–60.

117. Segal KR, Gutin B, Presta E, Wang J, Van Itallie TB. Estimation of human body composition by electrical impedance methods: A comparative study. J Appl Physiol. 1985;58(5):1565–71.

118. Ruiz L, Colley JR, Hamilton PJ. Measurement of triceps skinfold thickness. An investigation of sources of variation. Br J Prev Soc Med. 1971;25(3):165–7.

119. Burkinshaw L, Jones PRM, Krupowicz DW. Observer Error in Skinfold Thickness Measurements. Hum Biol. 1973;45(2):273–9.

120. Rothman KJ. BMI-related errors in the measurement of obesity. Int J Obes. 2008;32(Supplement):56–9.

121. Misra A, Soares MJ, Mohan V, Anoop S, Abhishek V, Vaidya R, et al. Body fat, metabolic syndrome and hyperglycemia in South Asians. J Diabetes Complications [Internet]. 2018;32(11):1068–75. Available from:

https://doi.org/10.1016/j.jdiacomp.2018.08.001

122. Okorodudu DO, Jumean MF, Montori VM, Romero-Corral A, Somers VK, Erwin PJ, et al. Diagnostic performance of body mass index to identify obesity as defined by body adiposity: A systematic review and meta-analysis. Int J Obes [Internet]. 2010;34(5):791–9. Available from: http://dx.doi.org/10.1038/ijo.2010.5

123. Gómez-Ambrosi J, Silva C, Galofré JC, Escalada J, Santos S, Millán D, et al. Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. Int J Obes. 2012;36(2):286–94.

124. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet. 2005;37(2):161–5.

125. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart Disease and Stroke Statistics—2011 Update: A Report From the American Heart Association RD on behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2011;123(4):18–209.

126. Lovejoy JC, De La Bretonne JA, Klemperer M, Tulley R. Abdominal fat distribution and metabolic risk factors: Effects of race. Metabolism. 1996;45(9):1119–24.

127. Mills HL, Patel N, White SL, Pasupathy D, Briley AL, Santos Ferreira DL, et al. The effect of a lifestyle intervention in obese pregnant women on gestational

metabolic profiles: Findings from the UK Pregnancies Better Eating and Activity Trial (UPBEAT) randomised controlled trial. BMC Med. 2019;17(1):1–12.

128. Murray-Davis B, McDonald H, Rietsma A, Coubrough M, Hutton E. Deciding on home or hospital birth: Results of the Ontario choice of birthplace survey. Midwifery [Internet]. 2014;30(7):869–76. Available from: http://dx.doi.org/10.1016/j.midw.2014.01.008

129. Jonas O, Roder D, Chan A. The association of low socio-economic status in metropolitan adelaide with maternal demographic and obstetric characteristics and pregnancy outcome. Eur J Epidemiol. 1992;8(5):708–14.

130. Kenny LC, Lavender T, McNamee R, O'Neill SM, Mills T, Khashan AS. Advanced Maternal Age and Adverse Pregnancy Outcome: Evidence from a Large Contemporary Cohort. PLoS One. 2013;8(2):1–9.

131. Zhang S, Cardarelli K, Shim R, Ye J, Booker KL, Rust G. Racial disparities in economic and clinical outcomes of pregnancy among medicaid recipients. Matern Child Health J. 2013;17(8):1518–25.

132. Butte NF. Carbohydrate and lipid metabolism in pregnancy: Normal compared with gestational diabetes mellitus. Am J Clin Nutr. 2000;71(5 SUPPL.):1256–61.

133. Catalano P, DeMouzon S. Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. Int J obesitty. 2016;39(4):642–9.

134. Bosaeus M, Hussain A, Karlsson T, Andersson L, Hulthén L, Svelander C, et al. A randomized longitudinal dietary intervention study during pregnancy: Effects on fish intake, phospholipids, and body composition. Nutr J. 2015;14(1):1–13.

135. Dodd JM, Kannieappan LM, Grivell RM, Deussen AR, Moran LJ, Yelland LN, et al. Effects of an antenatal dietary intervention on maternal anthropometric measures in pregnant women with obesity. Obesity. 2015;23(8):1555–62.