# Impact of infant feeding status on maternal body composition

By

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#### **Abstract**

**Background:** Overweight and obesity are related to a greater risk for health problems throughout life. Maternal overweight and obesity is related to complications during pregnancy that endanger the health of the mother and her developing fetus. Pregnancy is also a time when excessive weight can be gained leading the mother susceptible to an increased risk of long term overweight or obesity and entering subsequent pregnancies with a higher BMI. Breastfeeding has been shown to create a caloric deficit to promote weight loss, yet no study has assessed breastfeeding exclusivity at 3 months and maternal body composition at 6 months as well as breastfeeding exclusivity at 6 months and maternal body composition at 6 months.

**Purpose:** The purpose of this study was to explore the relationship between infant feeding status at three and six months post-partum and maternal body composition at six months post-partum.

Methods: Twenty-five mothers were included in this analysis. Mothers were included if they met the criteria of a BMI  $18.5 - 40 \text{ kg/m}^2$ , of a singleton pregnancy, healthy, completed a 3 month post-partum follow-up visit with breastfeeding questionnaire and a 6 month post-partum follow-up visit with breastfeeding questionnaire and Bod Pod test. Mothers were dichotomized into exclusive breastfeeding and non-exclusive breastfeeding. Maternal body composition was measured by air displacement plethysmography by the Bod Pod. ANOVA assessed the relationship between maternal breastfeeding status at 3 months and maternal body composition at 6 months as well as the relationship between maternal breastfeeding status at 6 months and maternal body composition at 6 months. Multiple linear regression assessed the relationship between post-partum weight loss 0-3 months and 3-6 months and maternal body composition at 6 months. Significance was determined at a level of p < 0.05.

**Results:** The mean age of the participants was 30.4 years, the mean pre-pregnancy BMI was  $26.6 \text{ kg/m}^2$ , and the mean total PPWL was  $11.6 \text{ kg} \pm 5.5 \text{ kg}$ . Differences were found between groups for the amount of total PPWL (exclusive:  $13.2 \pm 5.3 \text{ kg}$  and non-exclusive:  $8.4 \text{ kg} \pm 4.7 \text{ kg}$ ; p=0.017) and PPWL 3 to 6 months (exclusive:  $2.6 \pm 1.7 \text{ kg}$  and non-exclusive:  $0.06 \pm 3.2 \text{ kg}$ ; p=0.039). When examining breastfeeding status at 3 months, no between group (exclusive vs. non-exclusive) differences were found for maternal body composition. Similarly, when examining differences in breastfeeding status at 6 months, no differences in maternal body composition was found. Linear regression showed post-partum weight loss from 3 to 6 months predicted maternal %fat ( $\beta = 1.87$ ,  $R^2 = 0.22$ ; p = 0.010), FM ( $\beta = 0.608$ ,  $R^2 = 0.34$ ; p = 0.001) and FFM ( $\beta = 0.473$ , R2 = 0.19; p = 0.017).

**Conclusion:** No relationship was found between breastfeeding exclusivity assessed at 3 and 6 months post-partum and maternal body composition at 6 months post-partum. However, a relationship was found among PPWL 3 – 6 months post-partum and maternal % fat, FM, FFM at 6 months post-partum. Future research is needed to better support these relationships as well as to determine to mechanisms which influence them.

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## **Chapter 1: Introduction**

In the United States over one third of women are obese (1), over 50 percent of pregnant women are overweight or obese, and extremely obese women make up eight percent of the women who are of reproductive age (2). Overweight and obesity are related to a greater risk for health problems throughout life. Maternal overweight and obesity is related to complications during pregnancy that endanger the health of the mother and her developing fetus (2). Pregnancy complications in overweight and obese women include an increased of risk gestational diabetes mellitus (GDM) (3), hypertension (3), preeclampsia and post-partum weight retention (1). Further, offspring exposed to maternal overweight or obesity are at a greater risk of developing childhood obesity and chronic diseases (4, 5)

During pregnancy, body weight is gained to support appropriate fetal growth and development (6, 7). Maternal body weight increases due to increases in water, protein, fat and mineral (8, 9) that is gained in both the maternal and fetal compartments. Fat is gained and stored as an energy reserve to be utilized to support fetal growth and lactation (3) post-partum. However the amount of body fat gained varies depending on amount of gestational weight gain and pre-pregnancy BMI (10-12).

Contrary to what one might think, women who are of the under or normal weight categories are more likely to gain an excessive amount of weight during pregnancy (13). Additionally, they often retain a significant amount of weight gained during pregnancy leading to increased body weight in subsequent pregnancies or throughout adulthood (1). Studies have focused on providing resources to women in order to limit gestational weight gain to within the Institute of Medicine (IOM) recommendations (14), or to encourage post-partum weight loss by intervening with diet and exercise post-partum (1). Other maternal behaviors are related to greater post-partum weight loss (15-19) such as breastfeeding exclusivity and duration. Several

of these studies (15-19) have explored the necessary duration of exclusive or even mixed feeding (including both breastfeeding and formula feeding) to promote post-partum weight loss, however few studies have examined how breastfeeding relates to maternal body composition at six months post-partum.

## **Statement of Purpose**

The purpose of this study was to explore the relationship between infant feeding status at three and six months post-partum and maternal body composition at six months post-partum.

#### **Research Question**

Does maternal body composition (percentage body fat (%fat), fat mass (FM) and fat free mass (FFM)) differ by infant feeding status (exclusively breastfed and non-exclusively breastfed)

Specific aims and Hypotheses:

Aim 1: To determine if maternal body composition (% fat, FM and FFM) differs by infant feeding status at three months post-partum.

Hypothesis 1.1: Women who exclusively breastfeed will have lower %fat than women who do not exclusively breast feed.

Hypothesis 1.2: Women who exclusively breastfeed will have lower FM than women who do not exclusively breast feed.

Hypothesis 1.3: Women who exclusively breastfeed will have greater FFM than women who do not exclusively breast feed.

Aim 2: To determine if maternal body composition (% fat, FM and FFM) differs by infant feeding status at six months post-partum.

Hypothesis 1.1: Women who exclusively breastfeed will have lower %fat than women who do not exclusively breast feed.

Hypothesis 1.2: Women who exclusively breastfeed will have lower FM than women who do not exclusively breast feed.

Hypothesis 1.3: Women who exclusively breastfeed will have greater FFM than women who do not exclusively breast feed.

Aim 3: Explore the relationship between post-partum weight loss at 3 months and 6 months in relation to maternal % fat at 6 months.

Hypothesis 1.1: Women who have higher weight loss at 3 months and 6 months will have a lower % fat at 6 months.

#### **Chapter 2: Literature Review**

#### **Obesity rates**

Obesity rates have increased significantly over the past several decades (20-22) in the United States, causing concern. Extensive analysis of the obesity rates using data from NHANES III show that from 2011-2012 over one-third of the American youth were classified as being overweight or obese with 16.9% (4, 21) of this population falling in the obese category. Obesity prevalence increases with age; 8% (4, 21) of 2-5 year olds, 17.7% (4, 21) of the 6-11 year olds and 20.5% (4, 21) of 12-19 year olds were obese. These rates have increased significantly since 1980 when only 7% of children aged 6-11 years (4, 21) and 5% of adolescences aged 12-19 years (4, 21) were diagnosed with obesity. A similar increase was found among adults as well. The prevalence of overweight or obesity has increased from 48.5% to 69.0% (21, 23) among all adults over the age of 20. Currently obesity has reached a plateau over the years with 35.1% (21, 23) of adults having a BMI of 30 or greater. This is in contrast to the 16.4% obesity rate reported in 1980(4, 21).

Obesity can cause greater risk of developing threatening conditions at many stages throughout one's life. Entering pregnancy with an overweight or obese BMI is related to greater risk of developing harmful conditions such as preeclampsia or GDM (20, 21, 23). The 2002 National Survey of Family Growth (NSFG) surveyed women of childbearing potential to assess the incidence rate of a BMI >25 (23). Results showed that of the non-pregnant females aged 20 to 44 years old, 47.5% were overweight or obese (23) and the risk for obesity increased with age (23). In this population, one out of ten (20, 23) women met requirements for classification as either class II or III obesity. Nearly one-half (20, 23) of women enter pregnancy at either an

overweight or obese BMI classification. Among all pregnant women, regardless of age, race, or number of pregnancy, the obesity rate increased from 17.6% (20, 23) in 2003 to 20.5% (20, 24) in 2009. BMI classifications in pregnancy not only provide insight into a woman's probability of experiencing medical complications throughout the pregnancy, but they also can predict possible weight retention post-partum (13, 25, 26) and are used in determining the appropriate among of gestation weight gain (26).

#### Gestational weight gain recommendations

In the United States, the Institute of Medicine (IOM) is the governing body that develops and revises the recommendations for gestational weight gain (GWG). Over the years these recommendations have evolved (7) as research has exposed the negative effects of excessive weight gain during pregnancy in both mother and infant (7).

## Changes in GWG recommendations

Changes in GWG recommendations have evolved over the past 5 decades spanning from strict recommendations targeting the prevention of toxemia, difficult births and maternal obesity (7, 12, 27, 28) to infant health outcomes (12, 27). In the 1960s, obstetricians were advised to limit GWG to 11.3 kg at most (7), but preferred a lower GWG at 6.8 kg (7). Health care professionals recognized the severity of limiting GWG when rates of infant disability, morbidity and mortality increased (7, 11, 12, 27). These GWG recommendations were lifted in the 1970s and were not re-evaluated until the 1990s when researched showed a strong correlation between GWG and infant size, mental health and mortality (7). The IOM recommendations published in the 1990 report suggested a 12.5-18 kg total gain for women whose BMI was <19.8 kg/m², 11.5-16 kg for BMIs 19.9-26.0 kg/m², and 7-11.5 kg for >26.0-29.0 kg/m² BMIs (7). For women who were obese (BMI >29.0), a weight gain recommendation of <6.0 kg was made (7). While the

recommendations published in 1990 may not differ significantly from those used today, a revision was made by the IOM in 2009 to alter the GWG guidelines to match the BMI categories as defined by the World Health Organization (WHO) (7).

#### Current recommendations

Current IOM recommendations suggest that GWG be tailored to BMI categories established by the WHO and should promote positive outcomes for both mother and baby (7). Women whose BMI is <18.5 kg/m<sup>2</sup> are encouraged to gain 12.5-18 kg (29, 30), a BMI of 18.5-24.9 kg/m<sup>2</sup> suggests a weight gain of 11.5-16 kg (29, 30). Pregnant women with a pre-pregnancy BMI of 25-29.9 kg/m<sup>2</sup> are recommended to gain between 7 -11.5 kg (29, 30), and obese women with a pre-pregnancy BMI of >30 kg/m<sup>2</sup> should gain 5-9.1 kg during their pregnancy (29, 30). GWG outside of IOM recommendations, both low and high, also resulted in adverse fetal weights (3, 7).

Rates of adherence to weight gain guidelines

Weight gain guidelines published by the IOM in 2009 were thought to apply less strict guidelines on women than those originally published in 1960 (31) while targeting the prevention of both maternal and infant adverse events. However, studies (7, 27, 32) showed that approximately two-thirds of women were not adhering to these guidelines. In a 2013 study, 61% (33) of participants did not adhere to the GWG guidelines tailored to their pre-pregnancy BMI status. In 2015, a similar study produced supporting results with 68% (32) of women gaining outside of the guidelines, 20.9% (32) not gaining adequate weight and 47.2% (32) gaining excessively. When adjusting for pre-pregnancy BMI, women who were overweight or of the obese I BMI category had the highest prevalence rate of excessive GWG (64.1% and 63.5%, respectively) (32). This is not to say that women who were underweight or of normal weight did

not gain excessively as well; 20.1% of underweight women (27, 28, 32) and 37.3% of normal weight (27, 28, 32) women gained over the IOM recommendation for GWG. Gestational weight gain, especially excessive amounts, and maternal obesity are responsible for complications both during pregnancy and for maternal health in the long term (7, 27, 28, 32, 34).

## **Complications related to maternal obesity**

Women who enter pregnancy at either an overweight or obese BMI are at a higher risk for developing complications both during (25, 35-37) and after pregnancy (13, 17, 25, 29, 38-41). Developing complications such as preeclampsia (29, 42-45) and GDM (25, 35-37) in a woman's first pregnancy is a risk factor for developing these same complications in subsequent pregnancies. Additional studies (25, 37, 46, 47) have linked maternal obesity before and during pregnancy to higher post-partum weight retention.

## Complications during pregnancy

Maternal obesity creates complications in a woman's ability to conceive naturally (34, 48, 49) as well as maintain a viable pregnancy (34, 48, 49) independent of the conception method. Women who are overweight or obese and otherwise healthy are less likely to conceive naturally compared to their normal weight counterparts (50). In a study examining the obesity-fecundity (the probability of conceiving for a given menstrual cycle) association among normal, overweight and obese women, obesity had a significant impact (50) on a woman's ability to become pregnant. Those who were obese had an average of 5 months to pregnancy, while overweight and normal weight women had 4 months to pregnancy (50). The fecundablity was reduced by 8% and 18% for overweight and obese women respectively (50). Once a woman became pregnant, those who are overweight or obese experienced miscarriages at a higher rate (48) than women of normal weight. Khaskheli et al. (48), assessed the impact weight reduction in

obese women would have on the spontaneous conception rate as well as the miscarriage rate. With a mean decrease in BMI of 9.6 kg/m<sup>2</sup>, spontaneous conception was 41.17% (48) and the miscarriage rate was reduced by 16.66% (48). Thus, losing weight prior to conception can positively influence a woman's ability to conceive and maintain a healthy pregnancy.

However, should a woman enter pregnancy with an overweight or obese BMI, they are at greater risk for developing conditions such as preeclampsia (51). Preeclampsia affects 2-8% of all pregnancies (45, 51) and is a cause in the development of additional diseases affecting the mother and can ultimately lead to death (45, 51). Clinically, preeclampsia is diagnosed through hypertension and proteinuria occurring in or after the 20<sup>th</sup> week of gestation (45, 51). Preeclampsia is associated with many risk factors such as preeclampsia in previous pregnancies, maternal age (young or advanced), being of the African-American race, smoking and family history (45, 51). Many of these risk factors are non-modifiable, but one risk factor of growing concern is: obesity. There is a positive association with increasing pre-pregnancy BMI and the diagnosis of preeclampsia (45). Having a BMI in the overweight or obese categories increases a woman's chance of developing preeclampsia two, even three-fold over those with a normal BMI (44).

An increase in BMI not only has a positive association with the development of preeclampsia, but also with GDM. In a study conducted by Heude et al., 19% of women with who had a BMI of 30 kg/m<sup>2</sup> or greater developed GDM compared to 4% of women with a normal BMI (18.5-24.9 kg/m<sup>2</sup>) (3). Due to the health effects known to coincide with GDM such as insulin insensitivity and hyperglycemia (52), The Hyperglycemia and Adverse Pregnancy Outcome Study (HAPO) (52) examined the association between GDM and harmful events in pregnancy. Women who were diagnosed with GDM, but who were not obese and women who

were both obese and diagnosed with GDM delivered through primary cesarean section 23% and 28.7% of the time respectively, compared to 16% of women delivering through cesarean section who were classified as "No GDM, no obesity" (52).

Maternal obesity not only increases the risk of complications during pregnancy, but also relates to the delivery method (1, 29, 34). The cesarean section delivery rate is 20.7% (53) for women who have a BMI of 29.9 kg/m² or less, 33.8% for a BMI of 30-34-9 kg/m² and 47% (53) for a BMI of 35-39.9 kg/m². Delivery via cesarean sections can significantly decrease a mother's ability to successfully delivery vaginally in following pregnancies (35). Maternal obesity throughout pregnancy has the potential to negatively affect the mother during the gestational period, and subsequent pregnancy that could follow.

## Complications long term

Obesity in general can lead to the development of the metabolic syndrome (11, 12) which is an underlying cause of coronary heart disease (11). In pregnancy, the fluctuation in hormones produces many symptoms synonymous (11) to that of the metabolic syndrome. The inability to compensate for increased insulin resistance during pregnancy that results in the development of GDM puts a woman at an increased risk for developing type 2 diabetes later in life (12). Even subtle increases in fasting lipid levels, blood pressure and large vessel function (12) not only increases the future risk of diabetes development in the mother but also the development of coronary heart disease (12).

The development of preeclampsia at 30 weeks gestation or later (12), can have long-term health implications on the mother as well. Women who experience preeclampsia have a higher relative risk (risk ratio: 2.61) (54) of dying from coronary heart disease than those who only experienced hypertension (risk ratio: 1.90) (54). Ischeamic heart disease (relative risk 1.7) (54),

elevated lipid levels (54) and endothelial dysfunction (54) are all additional cardiovascular complications experienced long term due to maternal obesity, and are conditions that can decrease quality of life.

#### **Complications of Excessive Gestational Weight Gain**

Due to the necessity of gaining weight in pregnancy, this time period is also viewed as a high risk time period for women to gain large amounts of weight (28, 55, 56). A large gain in body weight without post-partum weight loss increases the likelihood of increasing their post-partum BMI. Depending on their pre-pregnancy BMI, this may move them from a normal BMI into an overweight or even obese BMI category (57). Excessive GWG throughout pregnancy puts women at risk for developing the same health complications (28, 55, 56) (preeclampsia, and GDM) as entering pregnancy with an overweight or obese BMI and is related to greater post-partum weight retention (9).

## Complications during pregnancy

Gestational weight gain outside of the IOM recommendations can put mothers at risk for the development of GDM and preeclampsia (26). Mothers who exceeded GWG by 28 weeks of gestation had higher HOMA indices ( $1.08 \pm 0.75$ , p=0.04) and higher concentrations of leptin ( $9.71 \pm 0.8$ , p<0.001) than in those who did not exceed IOM guidelines (12). Women who exceeded the IOM guidelines were also more likely to delivery via caesarean section (13% vs. 4%, P<0.0001) (12). Of those women who gained excessively in a first pregnancy, 65% (12) were likely to gain excessively in their second pregnancy.

## Long-term complications

Significant complications of excessive GWG are present beyond the pregnancy itself (58). Excessive GWG is associated with greater risk of post-partum weight retention and

experiencing adverse cardiometabolic events (58). These cardiometabolic events include obesity, central adiposity and higher systolic blood pressure (SBP) (58). At 7 years post-partum, waist circumference was greater among women who experienced excessive GWG in the first trimester (58) when compared to women who gained appropriately. Additionally, early GWG resulted in higher SBP (58) at 3 years post-partum.

The components of GWG (FM, FFM) are also risk factors for long-term maternal complications. Medium and high late GWG led to an increase in FM over 14 to 37 weeks gestation (risk ratio 2.72 and 5.56 respectively for second trimester; risk ratio 1.97 and 4.55 respectively for third trimester) (59) in comparison to women who were more conservative in GWG in the second and third trimesters. Excessive total GWG assessed at 37 weeks gestation was also associated with larger increases in maternal FM at 37 weeks gestation ( $\beta$  range for fat  $\Delta = 2.86$ -5.29 kg, p < 0.01) (59). While FM is necessary to supply energy for fetal growth as well as breastfeeding for the infant, FM is a contributor to obesity related cardiometabolic conditions.

## **Predictors of Post-partum Weight Retention**

Research exploring predictors of maternal weight following delivery may focus on either post-partum weight loss or PPWR. Within the literature, post-partum weight loss is typically the weight loss experienced up to 18 months post-partum, while PPWR is any weight retained from the 18 months forward. With more than approximately two-thirds of women (28) gaining above the IOM recommendations for GWG, pregnancy is viewed a critical window related to weight retention and development of long-term maternal obesity (13, 38). The risk for developing obesity following delivery can be combated by minimizing post-partum weight retention (PPWR). There are many predictors of PPWR however GWG and pre-pregnancy BMI are two of

the modifiable risk factors (3, 14, 60). Extensive data on PPWR is not available, but a large five-site, prospective cohort study found that 75% (57) of women were heavier at 1 year post-partum compared to their pre-pregnancy weight. In the study sample, 47.4% of women retained more than 10 lbs (57) and 24.2% retained more than 20 lbs at one year post-partum (57). *Infant feeding method* 

While research surrounding PPWR has spanned both short-term and long-term time points, studies have suggested that 6 months (15) and 1 year (57) post-partum are the most crucial for determining long term weight retention from GWG. These time points might coincide with the length of time a woman chooses to breastfeed, when they introduce solids, or completely wean their infant from breastfeeding. In a retrospective follow-up study (15), women who exclusively breastfed were compared to those who chose to mix feed (used both formula and breastfeeding) or formula feed for body weight retention at 3 and 6 months post-partum. After adjusting for covariates, there was no association (15) between feeding method and weight retention at 3 months post-partum. However, at the 6 month time point those who exclusively breastfed were 1.38 kg lighter than their formula fed counter parts (P≤0.0001) (15). An analysis was also completed to compare the mixed feeders to the formula feeders. Results found the mixed feeders retained 0.84kg less body weight (p=0.0002) (15) than the formula feeders. This suggests that any breastfeeding aids in weight loss when compared to women who formula feed. *Pre-pregnancy BMI and weight retention* 

There is a negative relationship between pre-pregnancy BMI and body weight retention; as pre-pregnancy BMI increases PPWR decreases (6, 13). This is likely a reflection of the IOM GWG recommendations; as pre-pregnancy BMI increases, the amount of recommended weight to gain decreases. A longitudinal study was conducted to evaluate the effect pre-pregnancy BMI,

GWG and PPWR had on women at 1 year post-partum and long term – 15 years following their last delivery (13). There were 563 women included in this study, 514 were considered normal weight (BMI<25kg/m²) and 45 were overweight (BMI>25kg/m²). After adjusting for parity and GWG, there was no significant difference of weight retention at 1 or 15 years post-partum among the two groups (13). While additional studies (6, 61) have not looked at the direct effect of pre-pregnancy BMI on PPWR, they did find an association between pre-pregnancy BMI, GWG and PPWR. These findings suggest that women who enter pregnancy at a higher pre-pregnancy BMI do not experience excessive GWG as frequently as those with a lower BMI (6, 13, 61), thus having less weight to lose post-partum.

#### GWG and PPWR

Excessive GWG has a strong positive relationship with PPWR (56). Women who gained over the IOM recommendations were heavier (8.4kg) (56) at the long-term follow up (average 8.3 years post-partum) than women who gained appropriately (6.5 kg; p=0.01) (56). This finding is also supported by He et al. (55) who found that women gaining excessively in pregnancy, among all BMI classes, were 4.05 times more likely to have substantial PPWR (4.55 kg above pre-pregnancy weight) at 1 year follow-up (55).

He at al. also evaluated GWG and PPWR in association with BMI. Among normal weight women the mean weight gain during pregnancy was 16.7kg, which exceeds the recommendations by 0.7 kg. These women then saw an average of 8.0 kg (55) retained at 3 months post-partum, 5.0 kg and 3.0 kg at 6 and 12 months post-partum, respectively (55). As supported by studies following women for longer periods of time, women who do not lose the weight gained during pregnancy within the first 6 to 12 months are less likely to return to their pre-pregnancy weight than those who did lose the weight gained (13, 55, 58, 62).

Assessing weight gain per trimester could be more beneficial when determining a mother's potential PPWR. Weight gained during the first trimester was more strongly associated with post-partum weight change (58) than the second or third trimesters. Results adjusted for parity showed the normal weight women did not return to pre-pregnancy body weight for as long as seven years, whereas obese women returned to pre-pregnancy weight within 3 years (58). The association between early GWG and PPWR is likely representative of the adipose tissue deposition rather than weight related to fetal growth, placenta or fluid since little growth of these tissues has occurred by the end of the first trimester. Kirkegaard et al (62) found a similar result in the Danish National Birth Cohort (DNBC). Weight measurements were obtained for prepregnancy weight, GWG, 6 months post-partum and 7 years post-partum. In normal weight women the amount of early pregnancy weight gained was positively related to long term postpartum weight retention. At the 7 year follow-up, for every 1-kg of weight gained over the average GWG among the study sample there was corresponding 0.18 kg greater weight retention (62). Additionally, for every 1-kg increase in weight retained at 6 months post-partum (62) there was a 0.48 kg increase (62) in body weight at the 7 year follow-up. The results of Linne et al. (13) support the findings of these studies suggesting that excessive GWG leads to higher weight retained past one year post-partum. In this specific study women who gained >15.6 kg retained and average of 9.9kg (13) at the 15 year follow-up, averaging 3.5-4.0 kg (13) more than those who experienced low and intermediate weight gains. These findings (13, 56, 62) stress the need for resources to help women gain appropriately throughout the pregnancy and return to their prepregnancy weight no later than one year post-partum.

#### Other predictors of PPWR

Gestational weight gain and pre-pregnancy BMI are not the only predictors of PPWR. Age, social support and psychosocial influence has an effect on a mother's ability to be successful at losing weight post-partum (8). Slotkin and Herbold (8) assessed the influence items identified as sources of stress including household income, marital status, time between delivery and returning to work, and social support such as child care had on post-partum weight loss (8). Women were classified as experiencing more weight loss success -"ability to lost 50-100% of post-partum weight by 6 months (8)" - and less weight loss success. It was concluded that women with mild to no stress were more successful at losing weight post-partum than those who indicated feeling moderate to severe stress in their lives (P=0.008). In conjunction with stress, those women who experienced moderate to severe depression were also less successful at achieving weight loss in the 6 month time frame than their counterparts. Women who returned to work sooner, in 4 months or less, were considered more successful at weight loss suggesting the social support and more help with child care would result in a higher weight loss success rate (8). Slotkins and Herbold also found that of the women observed, those who were more successful losing weight post-partum were also partaking in the behavior of breastfeeding.

#### **Maternal benefits of breastfeeding**

Current breastfeeding recommendations

The American Association of Pediatrics currently recommends exclusive breastfeeding for greater than or equal to 4 months and continuation of breastfeeding for an additional year (63). Studies have shown that women who adhere to these guidelines are more likely to retain less post-partum weight and even return to pre-pregnancy weight status (47, 60, 64-67). Intention, initiation and duration of breastfeeding are all influenced by many factors. Some of

these factors consist of socioeconomic status, education, relationship status (65), and prepregnancy BMI (68). Women who were obese or underweight before conception had a lower intention and initiation rate of breastfeeding and ceased this practice sooner than their normal weight counterparts (68).

## Current rates of breastfeeding

Rates of breastfeeding are on the raise in the United States. According to the CDC 2014
Breastfeeding Report Card, 79.2% (63) of women reported initiating breastfeeding, and was an increase of 2.7% (63) from the previous year. Of the women who initiated breastfeeding, 37.7% (63) continued to exclusively breastfeed at 3 months, with only 16.4% (63) of women continuing up to 6 months. Additionally, after initiating breastfeeding 49.0% (63) of women continued to breastfeeding to some extent at 6 months, and 27.% (63) continued until 12 months. A study assessing the adherence to the AAP breastfeeding recommendations found that of women who initiated breastfeeding, 29.0% (65) breastfed exclusively for ≥4months, and 20.3% (65) exclusively breastfed to greater than or equal to one year (65).

## Breastfeeding and post-partum weight loss

The positive influences breastfeeding has on maternal post-partum weight are strongly supported when mothers adhered to the AAP recommendations of exclusive breastfeeding to at least 4 months (66, 67, 69). In one study observing mothers and their breastfeeding behaviors to one year post-partum, those who practiced exclusive breastfeeding 6 months lost more weight than those who did not practice this exclusivity (mean weight loss -2.9kg vs -1.8kg p=0.004) (16). Another observational study showed both the short and long term effects of breastfeeding adherence. Breastfeeding as recommended resulted in an increased post-partum weight loss at 6 and 18 months women with a BMI of 18.5-34.9, independent of pre-pregnancy BMI (70).

Further, those who complied to the AAP recommendations of both exclusivity and duration of breastfeeding weighed 8.0 kg less at 6 years post-partum than those who never initiated breastfeeding (65). While these findings are small, they are positive and should encourage most women to try initiating breastfeeding.

While exclusive breastfeeding is the practice commonly preferred by pediatricians and used in recommendations, periods in which mothers may only partially breastfeed can also be beneficial for weight loss. Data from the DNBC analyzed multiple time points throughout pregnancy and lactation and their relationship to weight loss at 6 and 18 months post-partum and weight retention at 7 years post-partum. There was only a small inverse relationship observed between the numbers of weeks a mother breastfed and her weight at the 7 year follow-up. A mother retained 0.1 kg less (62) at the follow-up visit for every 10 week interval she breastfed longer than her counterparts. However, results from the 6 and 18 month measurements suggest that the 18 months post-partum period is the most crucial time for a mother to experience weight loss. An additional study (70) supports this finding and the findings of Sharma et al. When grouped by durations of <1 week of full and any breastfeeding, 1 month of full and 2 months of any breastfeeding, 3 months of full and 3 months of any breastfeeding, and 6 months of full and 6 months of any breastfeeding, women who were overweight or obese were less likely to breastfeed for the longer durations but also experienced more weight loss post-partum when engaging in breastfeeding for 6 months (70). Independent of GWG and pre-pregnancy BMI, should a woman follow the recommendations for breastfeeding they would retain approximately 2 kg less at 6 months post-partum and 0.5 kg less at 18 months post-partum than those who did not engage in breastfeeding (70).

Breastfeeding and maternal anthropometrics

Brewer et al. examined feeding methods - exclusive breastfeeding, combined feeding and exclusively formula feeding - and changes in skinfold thickness and overall percentage body fat measured as a summation of the skinfolds. Significant decreases in percentage body fat occurred in all feeding groups (71), but were experienced at different time points. There were decreases in skinfold measurements among all groups as well, but the pattern in which these losses occurred differed between groups. The formula feeding group experienced losses in percentage fat and skinfold thickness within the first 3 months following delivery. These changes occurred by 6 months for the breastfeeding and combined feeding groups (71) suggesting the need for a steady fat mobilization to support the nutrient needs for the infant.

Breastfeeding and maternal body composition

Experiencing weight loss due to lactation has proven to be beneficial to the components of body weight, FM and FFM and may be more indicative of preventing the development of cardiometabolic diseases than weight loss alone. Breastfeeding has been shown to have a positive effect on reducing maternal FM. In a study comparing mothers who exclusively breastfed (EBF) with those who utilized formula as well (MF), both experienced loss of FM at 12 weeks post-partum (64). While EBF mothers experienced more FFM loss in comparison to their MF counterparts, the percentage body fat lost in the EBF was more significant over time. These findings further support the idea that exclusive breastfeeding provides a protective effect against the development of cardiovascular and chronic diseases. Mothers who had breastfed their infants for this duration had lower total and relative body FM than those who did not (67). This protective effect was observed in the women 16-20 years after their last pregnancy. In this study

by Wiklund (18), energy intake and energy output through physical activity did not differ among the two groups, suggesting that the increased energy expenditure experienced during lactation was enough to produce a caloric deficit resulting in weight loss.

## **Summary**

Obesity among women of childbearing age is at a high and alarming rate. Many adverse events can occur before and during pregnancy that are related to a high BMI such as infertility, miscarriages, preeclampsia, and GDM. These events can also produce negative outcomes in the offspring. Targeting post-partum weight loss interventions that increase the caloric deficit may be beneficial. However, breastfeeding has been shown to provide this caloric deficit and provide a protective effect to both mother and baby. The benefits an infant experiences due to breastfeeding have been studied substantially unlike the maternal benefits which still require substantial research. It has been shown that weight loss can occur due to the high energy demands of the growing infant, but where the energy comes from is still to be determined. More studies are needed to determine the effect breastfeeding of any duration may have on maternal body composition and the protection in can provided against cardiovascular and chronic metabolic diseases.

## **Chapter 3: Methods**

#### Overview

This study used the cohort from the Thrasher study conducted at The University of Kansas Medical Center. The purpose of this study was to explore the relationship between breastfeeding status (exclusive, mostly breastfeeding and mixed feeding) and maternal body composition at 6 months post-partum.

## Sample

The study population was a subset of sixty-seven women who participated in the Thrasher observational study. Twenty-five women of a healthy (18.5-24.99 kg/m²) BMI, overweight (25.0-29.4 kg/m²) BMI and obese (29.5-40kg/m²) BMI categories completed both the 3 month follow-up visit and the 6 month body composition study visit. The original study was designed to investigate the factors affecting infant growth and body composition patterns.

## **Inclusion/Exclusion Criteria**

Inclusion criteria for the Thrasher study were: women between ages 18 and 45 years, a healthy (18.5-24.99 kg/m<sup>2</sup>) BMI, overweight (25.0-29.4 kg/m<sup>2</sup>) BMI and obese (29.5-40kg/m<sup>2</sup>) BMI before pregnancy, singleton pregnancy, English speaking, and were either seen by an Obstetrician at KU Hospital or were employees of the hospital or university.

Women were excluded if they were underweight (BMI < 18 kg/m²) before pregnancy, were under the age of 18 or over the age of 45, carried an infectious diseases, had diabetes mellitus, hypertension or preeclampsia, smoked or used drugs during pregnancy, were non-English speaking, expecting multiple infants, and were unwilling or unable to complete the birth visit or additional follow-up visits.

## **Setting**

The Thrasher study was conducted at the University of Kansas Hospital and Medical Center from September 2013 to March 2015.

#### **Ethics**

The study was approved by the Human Subjects Committee of the KU Medical Center (HSC#13126). Informed consent was signed by every individual. All staff members were trained before performing any procedures in the study.

#### **Procedures**

Women who saw an Obstetrician in KU Obstetrics and Gynecology Clinic were prescreened by reviewing their medical chart. Women who were eligible and between 12-18 weeks gestation were asked if they were interested in participating in a research study. The consent form was explained and signed by the participants at the first study visit. The original study included two visits during the gestational period and three visits post-partum. The visits of interest for this particular study were those conducted at 3 and 6 months post-partum. Maternal skin folds and breastfeeding questionnaires were completed at both of these visits, and maternal body composition was assessed by the Bod Pod at the 6 months visit.

#### **Instrumentation:**

## Analysis of maternal body composition by Bod Pod

The purpose of the Bod Pod was to assess total body weight (TBW), FM and FFM by air displacement plethysmography (ADP). ADP measures body volume and body mass. Based on the formula below, body density was calculated. Body density was used to measure FM and FFM. Body density of the individual was calculated by the following equation:

Body density = body mass / body volume

The Bod Pod system obtained body mass using an integrated digital scale. Body volume was measured when the subject sat inside the Bod Pod chamber. The body volume measurement took into consideration the air that came in contact with the subject's body surface area and the thoracic gas volume (TGV). TGV was determined by direct measure. Subjects were instructed to comply with the following:

- Avoid eating, drinking and exercising for at least 2 hours before the test.
- Wear minimal clothing, e.g. form fitting swimming suit, and remove accessories such as eye glasses and jewelry
- Wear a swim cap with all of the subject's hair placed inside

The operator entered body height and date of birth. The subject was asked to step onto the scale for her body weight measurement. After a volume calibration was completed, the subject entered the chamber to complete 2-3 body volume measurements. Finally, TGV was measured. The subject was instructed to breath normally through a tube that is connected to the Bod Pod. If the TGV test resulted in an abnormal value as determined by the Bod Pod, the TGV was repeated.

## **Anthropometrics**

#### **Body weight**

A digital scale connected to the Bod Pod software was used to measure body weight. The subject was dressed in light clothing and was barefoot. The subject stood in the center of the scale while the measurement is taken.

## **Body height**

Height was measured using a wall stadiometer. The subject was wearing no shoes and was instructed to stand with her back straight to the stadiometer. Subject's heels, buttock, shoulders and head were touching the wall, and facing straight ahead. The sliding headpiece was

placed upon the subjects head, touching the crown. The measurement was read and recorded to the nearest 0.1 cm.

#### **Questionnaires**

Participants were asked to complete infant feeding questionnaires at both 3 and 6 month follow-up visits. The following questions were used to assess breastfeeding status with breastfeeding being defined as "any method of feeding breast milk, i.e. feeding directly from breast or giving expressed breast milk in a bottle" and bottle-feeding being defined as "feeding formula milk using a bottle".

- Three month follow up: Which feeding methods did you use in the first three months?
  - Entirely breastfeeding
  - o Mostly breastfeeding with some bottle-feeding
  - o Equally breastfeeding and bottle-feeding
  - o Almost entirely bottle-feeding (only tried breastfeeding a few times)
  - o Entirely bottle-feeding (never tried breastfeeding)
- Six month follow up: Which feeding methods did you use in the last three months?
  - Entirely breastfeeding
  - Mostly breastfeeding with some bottle-feeding
  - Equally breastfeeding and bottle-feeding
  - Almost entirely bottle-feeding (only tried breastfeeding a few times)
  - Entirely bottle-feeding (never tried breastfeeding)
- Are you currently breastfeeding your baby? Yes/No
  - o If you are no longer breastfeeding, when did you stop?

#### **Chapter 4: Results**

This study was designed to determine if there was an association between breastfeeding exclusivity and maternal body composition (%fat, FM, FFM) assessed at 6 months post-partum. A second purpose was to investigate the relationships between PPWL from 0 to 3 months and 3 to 6 months to maternal body composition at 6 months post-partum.

#### **Subject Characteristics**

Twenty-five women completed all study visits. Table 1 presents the subject characteristics. The mean age of the participants was 30.4 years, the mean pre-pregnancy BMI was  $26.6 \text{ kg/m}^2$ , and the mean total PPWL was  $11.6 \text{ kg} \pm 5.5 \text{ kg}$ . For the entire sample, the majority of weight was lost by 3 months (9.9 kg  $\pm 5.1 \text{ kg}$ ) with an additional weight loss of 1.8 kg  $\pm 2.5 \text{ kg}$  from 3 to 6 months. Table 2 presents the subject characteristics by group (exclusive breastfeeding versus non-exclusive breastfeeding). At three months post-partum, 17 women were classified as exclusively breastfeeding and 8 were classified as non-exclusively breastfeeding. No differences were found between the groups for pre-pregnancy body weight or pre-pregnancy BMI. Differences were found between groups for the amount of total PPWL (exclusive:  $13.2 \pm 5.3 \text{ kg}$  and non-exclusive:  $8.4 \text{ kg} \pm 4.7 \text{ kg}$ ; p=0.017) and PPWL 3 to 6 months (exclusive:  $2.6 \pm 1.7 \text{ kg}$  and non-exclusive:  $0.06 \pm 3.2 \text{ kg}$ ; p=0.039). No difference was found between groups for PPWL 0 to 3 months (p=0.300).

## Maternal body composition and breastfeeding status

Reported in Table 3 are differences in maternal body composition based on breastfeeding status at 3 months and breastfeeding status at 6 months. Between 3 and 6 months, one person switched from exclusive to non-exclusive breastfeeding. Confounding variables included in the model were PPWL 0 to 3 months and PPWL 3 to 6 months. When examining breastfeeding

status at 3 months, no between group (exclusive vs. non-exclusive) differences were found for maternal body composition. Similarly, when examining differences in breastfeeding status at 6 months, no differences in maternal body composition was found.

## **Maternal Body Composition and PPWL**

The relationship between PPWL from 0 to 3 months and PPWL from 3 to 6 months to maternal %fat, FM, and FFM was assessed using linear regression. Maternal body composition was the dependent (outcome) variable and PPWL from 0 to 3 months and 3 to 6 months were the independent (predictor) variables. Only significant predictors were retained and PPWL from 0 to 3 months was non-significant and removed from the final models. Post-partum weight loss from 3 to 6 months predicted maternal %fat, FM and FFM. In the model predicting maternal %fat, for every one kg increase in PPWL from 3 to 6 months, maternal %fat decreased 1.87 %fat ( $\beta$  = 1.87,  $R^2$ =0.22; p = 0.010). In the model predicting maternal FM (kg), for every one kg increase in PPWL from 3 to 6 months, maternal FM decreased 0.61 kg ( $\beta$  = 0.608,  $R^2$ =0.34; p = 0.001). In the model predicting maternal FFM, for every one kg increase in PPWL from 3 to 6 months, maternal FFM decreased 0.47 kg ( $\beta$  = 0.473,  $R^2$ =0.19; p = 0.017). These results are summarized in table 4.

#### **Chapter 5: Discussion**

In this study, maternal breastfeeding exclusivity at both 3 and 6 months post-partum was assessed to explore their influence on maternal body composition components (%fat, FM, FFM) at 6 months post-partum. No difference was found for maternal body composition at 6 months post-partum based on breastfeeding status (exclusive vs. non-exclusive) at 3 or 6 months. However, exclusive breastfeeding women lost more body weight from 3 to 6 months and total weight since delivery. Further, we found PPWL from 3 to 6 months predicted maternal body composition at 6 months post-partum.

Prior studies have assessed breastfeeding status and post-partum weight loss, however this is the first study to our knowledge that explores PPWL at 0 to 3 months and 3 to 6 months and the difference in maternal body composition based on breastfeeding exclusivity. Prior studies have assessed breastfeeding status and changes in maternal skinfolds and overall percent body fat early in the post-partum period (between 4 and 8 weeks) (64, 67, 72). A study (64) has also assessed breastfeeding methods and changes in maternal body composition utilizing the Bod Pod at 12 weeks post-partum. These studies will be discussed.

#### Breastfeeding status and maternal body composition

The association between breastfeeding exclusivity and maternal body composition (%fat, FM, FFM) has received previous study, but lacks in consistency among methods and results (41, 64, 67, 72-74).

A summary of research using skinfolds to measure body composition

Sidebottom et al. assessed pregnancy-related factors and changes in maternal body fat using skinfold thickness in 557 women (72). Women in the study were classified as exclusively breastfeeding, exclusively bottle feeding or both breast and bottle feeding (indicating formula

use and not expressed breast milk). Skinfolds were taken at three sites (triceps, subscapular, and mid-thigh) and measured once every trimester and once again between 6 and 8 weeks post-partum (72). Sidebottom et al reported lower skinfold thickness at the three sites individually at 6 weeks post-partum in women who breastfed exclusively when compared with those who were classified as either breast and bottle feeding or those who exclusively bottle fed (72).

We found no difference in maternal body composition based on breastfeeding exclusivity however when predicting maternal body composition at 6 months, PPWL from 3 to 6 months was positively related. This is in contrast to the results by Sidebottom et al (72) who found a difference in skinfold thickness measured 6 to 8 weeks post-partum. There are several potential reasons for the discrepancy in findings between our studies. Sidebottom et al did have more participants, however they used skinfolds to assess maternal fat stores and we used the Bod Pod. Skinfold measures are more appropriately used to address distribution of fat versus assessing total body composition (75, 76). Additionally, the length to follow-up was shorter in the study conducted by Sidebottom et al and therefore cannot address the long term influence breastfeeding may have on maternal body composition that our study could have addressed. We measured maternal body composition at 6 months while Sidebottom et al measured skinfold thickness at 6 to 8 weeks (72).

A summary of research using the Bod Pod to measure body composition

Hatsu et al. (64) measured body composition using the Bod Pod at 2,4,8 and 12 weeks post-partum in mothers who were either exclusively breastfeeding or were mixed feeding. The 24 participants self-reported pre-pregnancy weight. The results found that both groups (exclusive and mixed feeders) had slightly higher FFM at 4 weeks when compared to the measure taken at 2 weeks. By the 12 week follow-visit the exclusive breastfeeding mothers had

lost FFM whereas the mothers who were classified as mixed feeding had gained FFM (p=0.004). A decrease in %fat was also found in the mixed feeding mothers over time as compared to their exclusively breastfeeding counterparts (p<0.05). While mixed feeding mothers had a lower %fat at 12 weeks post-partum, Hatsu et al did see a trend in the loss of %fat and FM across time in the exclusively breastfeeding mother when compared to mothers who were mixed feeding (64).

The results of our study are similar to those of Hatsu et al (64), and both address limitations of previous studies, yet a weakness of both is the small sample size. The use of repeated measures to assess body composition could be essential in determining if breastfeeding is beneficial to improving maternal body composition or returning it back to that of prepregnancy status. These measurements could also determine if and what duration of exclusive breastfeeding is needed to accomplish this as the studies differ on follow-up length.

#### **PPWL**

Research (1, 14, 47, 57, 66, 71, 72, 77) has shown that the critical time period for PPWL to occur is during the first 6 months. Weight loss during this time period is necessary in aiding post-partum weight retention and the prevention of long term obesity for the mother as well as entering subsequent pregnancies in a higher BMI category.

## Maternal body composition

Weight loss occurs as a result of a caloric deficit. However, a change in body composition depends upon what types of body tissues are being mobilized to help address the caloric deficit. Brewer et al (41) examined maternal weight changes, differences in fat deposits measured through skinfold thickness and percentage body fat using the sum of the skinfolds (triceps, subscapular and suprailiac) to estimate body density. The measurements were taken at three time points: 1 to 2 days after delivery, 3 months post-partum and 6 months post-partum.

Changes in weight, skinfold thickness and percentage body fat were computed at 0 to 3 months, 3 to 6 months and 0 to 6 months. Significant weight loss was experienced during the 0 to 3 months post-partum time frame, yet those who experienced a greater weight loss from 3 to 6 months also saw a greater decrease in their skinfold thickness from 0 to 6 months post-partum (41). While our study could not address a change in body composition, women who experienced greater PPWL during the 3 to 6 month time period were predicted to have lower %fat, FM and FFM at 6 months post-partum (41). This is similar to findings in our study.

## Breastfeeding status

Assessing the influence breastfeeding has on PPWL has been a challenge. Many studies (64, 65, 67, 69, 71, 72, 78, 79) agree with Butte et al (73, 74, 80) concluding that breastfeeding is energy demanding and therefore can produce a caloric deficit needed to assist in PPWL. Butte (73) concluded from a study of 45 lactating women that 156 kcal/d were provided by stored maternal tissue to help support the increased energy demand of breastfeeding. This caloric contribution was based upon energy intake from the diet, caloric equivalent of the milk, and change in body fat determined by water displacement (73).

Brewer et al (41) categorized mothers into breastfeeding, combined feeding and formula feeding to assess the influence breastfeeding on PPWL. A significant change in weight was observed in all three groups over the 6 month time period. However, women who were exclusively breastfeeding had a significant change in weight loss between 3 and 6 months when compared to the weight loss experienced by combined and formula feeders. These results found by Brewer et al (41) are supported by the results of our study suggesting that breastfeeding is a way to create a calorie deficit post-partum. Both Brewer and our study did also find that mothers who exclusively breastfeed had a higher GWG which could be a determinant of the higher

PPWL as there was more weight to be lost by those mothers. Future studies addressing breastfeeding and its influence on PPWL amongst women with similar GWG and pre-pregnancy BMI would need to be conducted to provide more support to a possible maternal benefit of lactation.

#### Limitations

This study has a few limitations. First, this study utilized a subsample from a parent observational study which enrolled women at 18 weeks of gestation and followed them until 6 months post-partum. Due to the length of the study, some women either withdrew or did not complete all of the study visits limiting the sample population. In conjunction with the length of the study adding additional Bod Pod testing could have increased subject burden, inhibiting or discouraging participation.

Assessment of breastfeeding status was obtained using a pre-constructed questionnaire that did not allow us to assess volume of breast milk expressed by the mothers and potential differences in energy expenditure. With the small sample size we were unable to classify breastfeeding into more than exclusive and non-exclusive. Additionally, collected dietary data was not available for analysis and therefore we could not account for and assess any difference in caloric intake that may be present between the two breastfeeding groups.

## Conclusion

In conclusion, we found no difference in maternal body composition at 6 months postpartum between in women based on breastfeeding exclusivity. Furthermore, PPWL throughout the 6 month post-partum period and its positive influence on maternal body composition was reinforced by the findings from our study. While our study did not assess the association between PPWL and maternal body composition between breastfeeding groups, lactation has been proven to create a caloric deficit through energy expenditure and therefore could be a method for mothers to either return or decrease their body fat percentage to that of their pre-pregnancy level. The findings of this study provide support for the current recommendations by the American Academy of Pediatrics of exclusive breastfeeding until at least 4 months of age and the World Health Organization of exclusive breastfeeding until 6 months of age.

Further questions can be developed from our results. While we did not find difference in groups based on breastfeeding duration and exclusivity, a larger study population and a test of maternal body composition at two time points would be appropriate to further examine these results. Body composition is beneficial in assessing health risks such as cardiovascular disease, metabolic syndrome, obesity and early death. Future studies should also examine the time point in which PPWL has the most positive effect on maternal body composition. These findings can translate into clinical application when determining resources and time points to provide mothers with education on how to live healthy lifestyles including physical activity and diet as well as breastfeeding support.

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# Appendix I

Table 1. Maternal Descriptive Statistics (n=25)

Maternal Characteristic	Mean ± SD
Age (yrs)	$30.4 \pm 3.3$
Weight (kg)	$73.8 \pm 17.0$
Height (cm)	$166.8 \pm 4.8$
BMI (kg/m <sup>2)</sup>	$26.6 \pm 6.5$
PPWL 0-3months (kg)	$-9.9 \pm 5.1$
PPWL 3-6months (kg)	-1.8± 2.5
Total PPWL (kg)	$-11.6 \pm 5.5$

**Table 2. Maternal Characteristics by breastfeeding status** 

	Exclusive	Non-exclusive	Significance
	Breastfeeding (n=17)	Breastfeeding (n=8)	
Maternal Characteristic	Mean ± SD		P-value
Weight (kg)	$71.5 \pm 15.5$	$78.7 \pm 20.0$	0.334
Height (cm)	$167.1 \pm 4.1$	$166.1 \pm 6.4$	0.617
Pre-BMI (kg/m <sup>2)</sup>	$25.7 \pm 6.0$	$28.6 \pm 7.6$	0.305
PPWL 0-3months (kg)	$-10.6 \pm 5.2$	$-8.3 \pm 4.7$	0.300
PPWL 3-6months (kg)	-2.6 ± 1.7	-0.1 ± 3.2	0.017*
Total PPWL (kg)	$-13.2 \pm 5.3$	$-8.4 \pm 4.7$	0.039*

Table 3: ANOVA to assess Maternal Body composition at 6 months

Breastfeeding status at 3 months			
	Exclusive Breastfeeding (n=17)	Non-exclusive	Significance
		Breastfeeding (n=8)	
%fat	$37.5 \pm 9.1$	$36.8 \pm 9.7$	0.878
FM (kg)	$29.8 \pm 12.3$	$28.2 \pm 13.1$	0.799
FFM (kg)	46.1 ± 4.7	$45.8 \pm 5.0$	0.870

## **Breastfeeding status at 6 months**

	Exclusive Breastfeeding (n=16)	Non-exclusive	Significance
		Breastfeeding (n=9)	
%fat	$38.3 \pm 9.2$	$35.5 \pm 8.7$	0.485
FM (kg)	$30.4 \pm 12.4$	27.3 ± 11.7	0.573
FFM (kg)	$45.4 \pm 4.7$	$47.0 \pm 4.5$	0.422

Table 4. Linear Regression Model predicting percentage body fat at 6 months postpartum

	β	$\mathbb{R}^2$	Significance	
Percentage body fat (%fat)				
PPWL 3 to 6 months	0.473	0.222	0.010*	
Fat mass (kg)				
PPWL 3 to 6 months	0.608	0.343	0.001*	
Fat free mass (kg)				
PPWL 3 to 6 months	0.473	0.190	0.017*	