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Mammary Gland Development

Adipocytes form a major proportion of the mammary gland and are necessary for proper gland development and proliferation (Machino, 1976; Landskroner-Eiger *et al.*, 2010). At puberty, alveolar ducts expand at the expense of the fat pad in the mammary gland (Hovey & Aimo, 2010; Macias & Hinck, 2012).

During pregnancy and lactation, adipocytes have a unique supportive function. Recently, it has been determined that mammary adipocytes de-differentiate gradually during gestation and almost disappear entirely during lactation allowing more space for milk production by the mammary alveolar epithelial cells (Wang *et al.*, 2018; Zwick *et al.*, 2018). Adipocytes closest to the mammary epithelial cells de-differentiate quicker than those farther away in the cleared fat pad (Hovey & Aimo, 2010; Lawson *et al.*, 2015). The alveoli expand at the expense of the fat pad almost entirely covering its area (Richert *et al.*, 2000). It is hypothesized that the adipocytes in the body mobilize their fat stores and provide for the mammary epithelial milk lipid production, which explains the reduction in size of the adipocytes during lactation (Flint & Vernon, 1998; Richert *et al.*, 2000; Cinti, 2018). The exact fate of adipocytes during the de-differentiation phase of lactation remains unknown (Wang *et al.*, 2018).

As milk production gradually decreases at weaning, adipocytes later grow rapidly in size by taking up excess milk lipids from the alveolar lumen and alveolar epithelial cells (Zwick *et al.*, 2018). This is referred to as a “refilling” process for the mammary gland adipocytes and it simultaneously occurs along epithelial cell regression (Zwick *et al.*, 2018).

Hormones:

Mammary Gland Function

Milk Composition

Human milk composition can differ depending on the timing it is sampled. One study found mean milk fat and energy content was higher in samples taken at night compared to the morning and there were no differences in carbohydrate and protein levels (Maron-Lev et. al, 2015). Milk composition can also differ by the type of milk that is being secreted; foremilk or hindmilk. Foremilk is the milk that is drawn first during feeding and hindmilk is secreted during the end of a feeding. Studies have found that their compositions differ, and because of this it is important that the timing of sampling be noted in our study. Total protein content was higher in hindmilk than foremilk and foremilk was higher in total free fatty acids (Sadelhoff et. al, 2018). Triglycerides were also found to be higher in hindmilk (Karatas et. al, 2011).

*Milk composition varies highly throughout lactation, between feeds, and during the day. In the first few days postpartum, the milk, called colostrum, is very thick and yellowish and is rich in immunoglobulin proteins to support the infant’s immunity. Colostrum has a caloric energy value of 58 Kcal/dL.*

*Fat comprises the majority of the calories in colostrum at 42.03% (2.9 g/dL) of the calories mainly in the form of triglycerides, carbohydrates account for 34.14% (5.3 g/dL) of the calories mainly in the form of lactose, and 23.83% (3.7 g/dL) of the calories are from protein (Godhia & Patel, 2013).*

*After the first few days of lactation, the milk becomes whiter and less thick due to an increase in lactose and a decrease in fat content. The milk produced after the first few days and until two weeks postpartum is classified as transitional milk. At two weeks postpartum, the milk fully matures and contains about 88% water and 65-70 Kcal/dL derived from the carbohydrates, protein and fat in the milk. The calories of the milk are derived from its macronutrients solid content comprising of 45% carbohydrates (6.7-7.8 g/dL) mainly in the form of lactose, about 7% protein (0.9-1.2 g/dL) mainly as whey protein or casein, and about 48% fat (3.2-3.6 g/dL), mainly in the form of triglycerides (Ballard & Morrow, 2013).*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Colostrum Macronutrient** | Fat (mainly TG) | CHO (mainly lactose) | Protein (mainly immunoglobulins, whey, casein) | Total |
| **Solid g/dL (g/100mL)** | 2.9  (24.37% of total solid) | 5.3  (44.54% of total solid) | 2.9  (31.09 % of total solid) | 11.9 |
| **Energy (Kcal)** | 26.1  (42.03% of total energy) | 21.2  (34.14% of total energy) | 14.8  (23.83% of total energy) | 62.1 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Mature Milk Macronutrient | Fat (mainly TG) | CHO (mainly lactose) | Protein | Total |
| Solid g/dL (g/100mL) | Range: 3.2-3.6  Average: 3.4 (29.05% of total solid) | Range: 6.7-7.8  Average: 7.25 (61.97% of total solid) | Range: 0.9-1.2  Average: 1.05 (8.97% of total solid) | Range: 10.8-12.6  Average:11.7 |
| Energy in Kcal | Range: 28.8-32.4  Average: 30.6 (47.96% of total average energy) | Range: 26.8-31.2  Average: 29 (45.45% of total average energy) | Range: 3.6-4.8  Average: 4.2 (6.58% of total average energy) | Range:59.2-68.4  Average: 63.8 |

Milk Fat Synthesis

Lipids, almost exclusively in the form of triglycerides, are synthesized in the smooth endoplasmic reticulum by de novo synthesis from available glucose, or they are derived from maternal diet or fatty acids from adipose tissue stores (Anderson *et al.*, 2007; McManaman, 2009; Rezaei *et al.*, 2016). The mechanisms by which lipids are packaged and transported into the milk remain elusive (McManaman, 2009).

Milk Protein Synthesis

Proteins are synthesized in the rough endoplasmic reticulum of the alveolar epithelial cells (Anderson *et al.*, 2007; Rezaei *et al.*, 2016).

Milk Carbohydrate Synthesis

Lactose is synthesized in the Golgi of the alveolar epithelial cells (Anderson *et al.*, 2007; Rezaei *et al.*, 2016).

Effect of Sex on Milk Composition

According to various studies there is not a clear relationship between sex of a child and the milk composition of a mother. In one study, there was no siginificant difference in milk composition, in terms of fat, protein, sugar, and energy by infant sex (Quinn, 2013). In contrast, another study found that women who delivered daughters had a higher concentration of fat in their breastmilk and mothers who had sons had higher salt levels in their breast milk (Hosseini et. al, 2020). Fat concentration was also seen to have been altered based on infant sex and socioeconomic status. Mothers who were economically sufficient produced a higher concentration of fat for their sons than daughters, and the opposite was seen for mothers who were more economically disadvantaged in Northern Kenya (Fujita et. al, 2012). Another study done with mothers in Massachusetts also found higher energy content in the breast milk of mothers of male infants (Powe et. al, 2009). Female infants were also found to be associated with lower carbohydrate and lower calorie content in human milk compared to males (Hahn et. al, 2016).

Insulin levels were higher in obese mothers nursing female infants and male infants compared to normal weight mothers nursing female infants and male infants, respectively (Fields et. al, 2016).

Why Breastfeeding Matters

Exclusive breastfeeding is used by the World Health Organization (WHO) as a strategy in reducing the burden of infant mortality and control of infection globally (Binns et. al, 2016). It is recommended by WHO that infants are exclusively breastfed up to the completion of 6 months and can be continued until the infant is at least 2 years old (Mosca and Gianni, 2017). The health benefits of breastfeeds for infants and mothers is extensive. For lactating women, reports show that they seek care less often, have lower frequency of respiratory, cardiovascular, and gestational diseases, and fewer symptoms related to emotional problems (Liuz and Leda, 2018). For infants, breastfeeding can have short-term and long-term effects. Short term effects include protection against infectious disease, protection against the risk of diarrhea and respiratory infections, reduced risk of otitis media, and reduced risk of malocclusion (Musca and Gianni, 2017). Long term effects include a reduction in the development of Type 2 Diabetes, development of obesity or overweight, and leukemia (Musca and Gianni, 2017). There have also been signs of increased cognitive outcomes in infants who were breastfed (Musca and Gianni, 2017).

Components of Breastmilk and Why They Matter

*Fat comprises the majority of the calories in colostrum at 42.03% (2.9 g/dL) of the calories mainly in the form of triglycerides, carbohydrates account for 34.14% (5.3 g/dL) of the calories mainly in the form of lactose, and 23.83% (3.7 g/dL) of the calories are from protein (Godhia & Patel, 2013).*

*After the first few days of lactation, the milk becomes whiter and less thick due to an increase in lactose and a decrease in fat content. The milk produced after the first few days and until two weeks postpartum is classified as transitional milk. At two weeks postpartum, the milk fully matures and contains about 88% water and 65-70 Kcal/dL derived from the carbohydrates, protein and fat in the milk. The calories of the milk are derived from its macronutrients solid content comprising of 45% carbohydrates (6.7-7.8 g/dL) mainly in the form of lactose, about 7% protein (0.9-1.2 g/dL) mainly as whey protein or casein, and about 48% fat (3.2-3.6 g/dL), mainly in the form of triglycerides (Ballard & Morrow, 2013).*

The importance of these various macronutrients is extensive and are key components of why breastmilk is so beneficial for infants. Proteins act as carriers for other nutrients, prompte gut development and nutrient absorption, and possess immune and antimicrobial activity (Mosca and Gianni, 2017). Carbohydrates in breastmilk are primarily in the form of the disaccharide lactose and are crucial for the development of the central nervous system (Mosca and Gianni, 2017). Human milk oligosaccharides (HMO) serve as probiotics and are also protective against necrotizing enterocolitis (NEC) (Mosca and Gianni, 2017). Lipids represent the major source of energy (44%) in human milk. They can be found in the form of dispersed fat globules where they serve as bioactive factors (Mosca and Gianni, 2017).

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The importance of these various macronutrients is extensive and are key components of why breastmilk is so beneficial for infants. Proteins act as carriers for other nutrients, prompte gut development and nutrient absorption, and possess immune and antimicrobial activity (Mosca and Gianni, 2017).  Longer lactation periods were associated with slightly lower protein content and calorie content in human milk (Hahn et.al, 2016). Protein levels during the day are also highly predictive of protein levels at night (Maron-Lev et. al, 2015). Foremilk and hindmilk have also been shown to have differing levels of protein, with high total protein content in hindmilk (Sadelhoff et. al, 2018). Protein content has been shown to decrease by at least 25% between 1 and 6 months of lactation (Allen et. al, 1991).

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Lipids represent the major source of energy (44%) in human milk. They can be found in the form of dispersed fat globules where they serve as bioactive factors (Mosca and Gianni, 2017). The concentration of fat and energy in human milk can vary based on the time of day, where it was found that milk fat and mean energy content was higher in the evening compared to the morning (Maron-Lev et. al, 2015). Fat content and energy contents have also been found to be correlated with the duration of lactation, where higher levels are found for longer lactation durations (Mandel et. al, 2005).This corresponds with the findings that fat content increased with breast emptying (Saarela et. al, 2007) Between foremilk and hindmilk, there seems to be compelling evidence that hindmilk has higher triglyceride levels than foremilk (Karatas et. al, 2011). Because of these findings, it is imperative to take into account the timing of our samples as well as whether or not the sample included foremilk and hindmilk or one or the other.

In a study done with Rhesus Macaques, it was found that primiparous mothers had less bodily resources available for lactation compared to multiparous mothers and therefore showed poorer lactation performance (Hinde, 2009). The effect of parity in our analysis did not show the same effect, but it is a covariate that may need to be studied further.

**Effect of Sex On Fat Content**

Our findings indicate that there was no difference in fat content between males and females at two weeks and two months. However, previous studies have found varying results. Due to the variation in lipid content depending on the timing of the sample, findings from other studies may not coincide with the procedures we took. Quinn and colleagues found no difference in milk composition between infant sex (2013), however Powe and collegues found that mothers carrying males had higher milk energy density compared to females (2009). Other studies showed that socioeconomic status of the mothers altered the lipid content of the breastmilk in relation to the sex of the child, however due to the stark differences in population, we do not believe this is finding can be drawn to our study (Fuijita et. al, 2012).

**Effect of Sex on Protein Content**

In our study, we found that protein content in human breast milk was higher among males than females and decreased in concentration from 2 weeks to 2 months. Sadelhoff and colleagues found that there was a sex difference between males and females, however, females had higher protein contant in the first 3 months compared to males (2018).

**Effects of Sex on Carbohydrate Content**

Our study found that there was no difference between males and females and carbohydrate content between two weeks and two months. However, in another study, Carbohydrate content was associated with infant sex, where females had a lower concentration compared to males (Hahn et. al, 2016). However, women carrying males were found to increase their intake of carbohydrates by 9.2% (Tamimi et. al, 2003).

**Energy Content**

Within our analysis there was no difference between the energy content of breastmilk between males and females.As previously discussed, a majority of energy content is in the form of lipids, therefore many trends seen with lipid content are similar to that of energy content. There was one study that had similar findings to ours, where there was no association between infant sex and energy content of breast milk (Quinn, 2013). However, in one study done with macaques, mothers of sons were shown to produce higher energy dense milk compared to mothers of daughters, however lower milk yield (Hinde, 2009). Breast Milk in human males was also shown to have higher energy density (Powe et. al., 2009). Women carrying males were also shown to have higher energy intake compared to women carrying infants (Tamini et. al, 2013) <https://www.imedpub.com/articles/biochemical-differences-in-human-breastmilk-contents-according-to-infants-gender.php?aid=23183> i think this fits here too, will add it to the table

Daily and colleagues explained that approximately 70% of the variance in fat content could be explained by the degree of breast emptying and about 41-95% of the variance for the fat content of the milk was explained by this factor for the individual breasts (1993).

**Sex specific hormones and their potential effect on milk- placental lactogen, estrogen, placental growth hormone, prolactin -probs discussion**

There are many hormones that can impact human breast milk synthesis and lactation; including reproductive hormones (estrogen, progesterone, placental lactogen, prolactin, and oxytocin), metabolic hormones (growth hormone, corticosteroids, thyroid hormones, and insulin), and mammary hormones (growth hormone, prolactin, parathyroid hormone-related peptide, and leptin) [(Neville et al., 2002)](https://www.zotero.org/google-docs/?Kseja2). Certain hormones are associated with certain aspects of mammary development [(Neville et al., 2002)](https://www.zotero.org/google-docs/?vZVQdQ). Ductal Morphogenesis is primarily driven by estrogen and growth hormone [(Neville et al., 2002)](https://www.zotero.org/google-docs/?8KzC6N). During alveolar morphogenesis there are two stages of lactogenesis. Lactogenesis 1 begins during mid pregnancy and in humans the appearance of ɑ-lactalbumin in the blood is the signal of onset [(Neville et al., 2002)](https://www.zotero.org/google-docs/?j5CgBT). Progesterone, placental lactogen, and corticosteroids appear to play a role in this stage [(Neville et al., 2002)](https://www.zotero.org/google-docs/?ZyJgto) Some of these hormones have greater effects than others. Prolactin is found to have a significant effect on the initiation and maintenance of breastmilk production [(Powe et al., 2011)](https://www.zotero.org/google-docs/?qy7kd1).

[**link**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3387861/#:~:text=In%20vitro%2C%20prolactin%20increases%20the,key%20regulator%20of%20lactose%20synthesis.&text=Prolactin%20also%20is%20involved%20in,lactose%20levels%20in%20mature%20milk.)

[**Link**](https://www.sciencedirect.com/science/article/abs/pii/S0301622605002812)

[**Link**](https://pubmed.ncbi.nlm.nih.gov/12160086/)

**Methods**

Recruitment

Methods have been described elsewhere PMID: [32074402](https://www.ncbi.nlm.nih.gov/pubmed/32074402). Briefly, “mother–infant dyads were recruited after written informed consent was obtained from mothers for their own participation and assent on behalf of their infant. Mothers were given detailed instructions for milk collection. Maternal demographics were obtained through paper survey with health history obtained through medical record review. Infant characteristics, growth measurements, and nutrition source were obtained through medical record review at birth and well child appointments at 2 weeks and 2 months~~, and 6 months.~~ Participants were given a stipend for their involvement. The Institutional Review Board of the University of Michigan (HUM00107801) and Saint Joseph Mercy Hospital (HSR ‐17‐1686) approved this study protocol.”

Participants

Sixty-four “~~Fifty‐five~~ dyads were enrolled peripartum while admitted for birth at two participating hospitals in Ann Arbor, Michigan, USA.” Milk samples were obtained from sixty-four participating mother-infant dyads at two weeks postpartum. Forty-four milk samples were obtained from participating mother-infant dyads at two months postpartum. Three dyads opted out of the study. Dyads were categorized on the basis of infant sex (male or female). ~~maternal pre‐pregnancy body mass index (BMI) into normal weight (NW; BMI <25.0 kg/m2) and overweight or obese (OW/OB; BMI ≥25.0 kg/m2).~~ “Pre‐pregnancy BMI was obtained through the medical record by obstetrics recorded pre‐pregnancy weight or early first trimester weight with BMI then calculated using recorded height. Inclusion criteria included maternal age > 18, gestational age ≥ 35 weeks, and healthy singleton birth with no maternal diabetes (WE HAVE 6 GDM CASES). “Dyads were enrolled if the mother intended to breastfeed but not excluded if formula was supplemented. Infant growth trends and milk composition associations with growth were excluded for those receiving exclusive formula feedings.”

Milk Collection and Analysis

Milk collection is describes elsewhere PMID: [32074402](https://www.ncbi.nlm.nih.gov/pubmed/32074402). Briefly, “samples were collected by mothers at home on the morning of their infant's physician visit near 2 weeks of life, on average at postpartum day 16. ~~This time point was selected given expected improvement in milk supply to allow for milk in transition to mature milk to be provided without limiting the infant supply.~~ Mothers collected nonfasting milk between 8:00 a.m. and 10:00 a.m. at least 2 hr after feeding their infant. Mothers provided milk by hand expression or pumping emptying an entire single breast. The method of expression and which breast to empty were based on maternal preference. Milk was expressed into a container then inverted to mix prior to transfer of 25 ml into glass vials in 5‐ml aliquots. Samples were stored in mother's home freezer then transported to clinic on ice for storage at −20°C for less than 1 week before transport on ice for final freezing at −80°C. Prior to analysis, samples were thawed on ice. Milk collection protocol was based on published milk collection with modifications as described for our study design and population (Fields & Demerath, [2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7296794/#mcn12979-bib-0013)). Milk analysis was performed using mid-infrared spectroscopy macronutrient analyser (MIRIS HMA™, Uppsala, Sweden) and is described in detail elsewhere (PMID: [32074402](https://www.ncbi.nlm.nih.gov/pubmed/32074402))

2.5. Infant growth measurements

“Infant anthropometrics were obtained by medical record review using birth and paediatric well‐child routine visits at birth, 2 weeks, and 2 months ~~and 6 months~~. Age‐ and sex‐specific *Z*‐scores for weight‐for‐age (WFA), length‐for‐age (LFA), head circumference‐for‐age (HCA), weight‐for‐length (WFL), and BMI using the World Health Organization (WHO) growth standard for infants 0 to 2 years of age were extracted (WHO Multicentre Growth Reference Study Group, [2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7296794/#mcn12979-bib-0056)).”

Statistical Methods

The data were analyzed using RStudio software (version 1.3.1073, 2009-2020). Initially, variables were checked for normality using Shapiro-Wilk test for continuous variables. Student’s *t*-tests or Mann-Whitney U testswere used for continuous variables as appropriate. Chi-squared tests were used for categorical variables as appropriate. Linear-mixed-effects models were used to assess the relationship between infant sex and milk composition, maternal characteristics, and time postpartum. Our initial model included sex of the infant as a predictor accounting for time postpartum and looking at fat, carbohydrates, protein, and milk energy content as outcomes. The final model included interaction between infant sex and time postpartum. A p-value of less than 0.05 was considered significant.

**Results**

Maternal Characteristics

Characteristics of the sixty-four enrolled women at two weeks postpartum are presented in Table 1. There were nos significant differences in maternal characteristics of women who delivered females and women who delivered males.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variables/ Sex of Baby** | | **Females** | **Males** | **Combined** | **p-Value** |
| **Maternal Age** | **n** | **n=35** | **n=28** | **n=63** | p=0.22  (TT) |
|  | 32 ± 0.689 | 30.7 ± 0.736 | 31.4 ± 0.506 |
| **Health Condition** | **n** | **n=35** | **n=28** | **n=63** | p=0.091  (CT) |
| Healthy | 29  82.86% | 19  67.86% | 48  76.19% |
| Gestational Diabetes | 2  5.71% | 4  14.29% | 6  9.52% |
| Polycystic Ovary Syndrome | 4  11.42% | 5  17.86% | 9  14.29% |
| **Gestational Age (Weeks)** | **n** | **n=35** | **n=28** | **n=63** | p=0.514 (MWT) |
|  | 39.8 ± 0.163 | 39.6 ± 0.181 | 39.7 ± 0.12 |
| **Parity** | **n** | **n=32** | **n=28** | **n=60** | p=0.241  (CT) |
| 1 | 14  43.75% | 13  46.43% | 27  45% |
| 2 | 13  40.63% | 12  42.86% | 25  41.67% |
| 3 | 4  12.5% | 3  10.71% | 7  11.67% |
| 5 | 1  3.13% | 0  0% | 1  1.67% |
| **Maternal BMI** | **n** | **n=33** | **n=27** | **n=60** | p=0.666 (MWT) |
|  | 27.0 ± 1.03 | 27.4 ± 1.16 | 27.2 ± 0.746 |
| **Ethnicity** | **n** | **n=35** | **n=28** | **n=63** | p=0.259  (CT) |
| Caucasian | 23  65.71% | 20  71.43% | 43  68.25% |
| African American | 3  8.57% | 2  7.14% | 5  7.94% |
| Asian | 4  11.43% | 3  10.71% | 7  11.11% |
| Latino | 3  8.57% | 3  10.71% | 6  9.52% |
| Other | 2  5.71% | 0  0% | 2  3.17% |
| **Delivery Method** | **n** | **n=35** | **n=28** | **n=63** | p=1  (CT) |
| Vaginal | 22 | 19 | 41 |
| Caesarean Section | 13 | 9 | 22 |
| **Education** | **n** | **n=31** | **n=28** | **n=59** | p=0.22  (CT) |
| High School or Less | 2  6.45% | 1  3.57% | 3  5.08% |
| Some College Credit, No Degree | 4  12.9% | 0  0% | 4  6.78% |
| Associates or Vocational | 0  0% | 3  10.71% | 3  5.08% |
| Undergraduate Degree | 12  38.71% | 9  32.14% | 21  35.6% |
| Graduate or Professional Degree | 13  41.94% | 15  53.57% | 28  47.46% |
| **Household** **Income** | **n** | **n=29** | **n=27** | **n=56** | p=0.199  (CT) |
| <60 K | 5  17.24% | 7  25.93% | 12  21.43% |
| >60 K | 24  82.76% | 20  74.07% | 44  78.57% |

Table Legend for Statistical Tests:

CT: Chi-squared test

MWT: Mann-Whitney U test

TT: Student’s t-test

WT: Welch’s t-test

Infant Characteristics

Pending- lindsay will send Noura will analyze- lindsay will send as of November

Milk Composition

Milk fat, carbohydrate, protein, and energy content are represented in Table/Figure 2.

Add the table here for 2 wks and 2 months comp- Or bar graph?