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## **Impact of Maternal Nutrition and Demographic Factors on Breast Milk Macronutrient Composition in Thai Mothers of Premature Infants**

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**IMPACT OF MATERNAL NUTRITION AND DEMOGRAPHIC FACTORS ON  
BREAST MILK MACRONUTRIENT COMPOSITION IN THAI  
MOTHERS OF PREMATURE INFANTS**

A dissertation Presented

By

SUKANYA KANKAEW

Submitted to the Graduate School of  
The University of Massachusetts Amherst in partial fulfillment of  
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DOCTOR OF PHILOSOPHY

September 2024

College of Nursing

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## **DEDICATION**

To my beloved fathers, you were my guiding light and greatest source of inspiration. Your unwavering support and love have been the foundation of everything I have achieved today. Although you are no longer with me, your presence remains in my heart, guiding me every step of the way. This dissertation is dedicated to you, with all my love and gratitude. Thank you for believing in me and for being my eternal source of strength.

To my dearest mothers, your unwavering love, support, and encouragement have been the bedrock of my journey. You have been my confidant, my cheerleader, and my greatest source of strength. This accomplishment would not have been possible without your endless sacrifices and belief in me. With all my heart, I dedicate this dissertation to you. Thank you for being my guiding star and for always being there for me.

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## **ABSTRACT**

# **IMPACT OF MATERNAL NUTRITION AND DEMOGRAPHIC FACTORS ON BREAST MILK MACRONUTRIENT COMPOSITION IN THAI MOTHERS OF PREMATURE INFANTS**

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Premature infants require higher nutrient intake to support their growth and development. Consequently, fortifying breast milk has become standard care to meet these infants' nutritional needs. However, the macronutrient composition of breast milk is highly variable among mothers and throughout lactation, leading to challenges in managing nutrient content. Understanding the factors that may affect changes in breast milk macronutrients should be considered.

This study aims to identify factors affecting macronutrient levels in breast milk from mothers of preterm infants in Thailand by examining maternal nutrition and demographic factors. A non-experimental correlational study design was employed to investigate how these factors influence macronutrient components in mature milk. Forty-seven mothers who gave premature birth were selected through convenience purposive sampling. Breast milk samples were collected at two-time points: during 1-2 weeks of lactation (time point 1) and 3-4 weeks of lactation (time point 2). Milk samples

were analyzed using the MIRIS human milk analyzer (HMA). Maternal dietary intake was assessed using 24-hour dietary food recall records from two non-consecutive days and food frequency questionnaires (FFQs). Maternal body mass index (BMI) was measured using current body weight and height.

The findings revealed significant changes in breast milk carbohydrates and protein levels from colostrum and transitional milk to mature milk (carbohydrates:  $Z = -3.511$ ,  $p < .001$ ; proteins:  $Z = -5.155$ ,  $p < .001$ ). However, fat and energy levels did not differ significantly (fat:  $Z = -1.96$ ,  $p = .05$ ; energy:  $Z = -1.498$ ,  $p = .134$ ). Multiple linear regression analysis showed a significant positive association between breast milk macronutrients and maternal dietary intake, including daily intake of carbohydrates, fats, and protein. However, no association was found between maternal BMI and breast milk macronutrients. Among maternal demographic factors, only the mode of delivery significantly influenced breast milk macronutrient levels. These findings support that maternal factors, particularly dietary intake, can affect the nutritional value of breast milk. Monitoring and modifying these related factors may improve breast milk macronutrient content for preterm infants.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background of the problem and significant of study

Premature infants are a vulnerable group with higher mortality and morbidity rates due to their underdeveloped body systems compared to healthy-term infants (Cao et al., 2022). The average rate of premature birth is 10.6% worldwide, ranging from 8.7% to 13.4% of infants born across regions, with an increasing trend in most African and South Asian countries (Chawanpaiboon et al., 2019). In Thailand, it is estimated that more than 700,000 infants are born prematurely, and of these, 9.4% have a birth weight less than 2,500 grams (low birthweight). Low birthweight leads to risk of severe complications including difficulty feeding, impaired body temperature regulation, and poor respiratory and immunological functions (Ministry of Public Health, 2016; National Statistical Office and United Nations Children's Fund, 2016). Preterm birth complications are the leading cause of death in children under the age of 5 years old worldwide and caused almost 1 million deaths in 2015 (World Health Organization, 2018). However, several studies show that incidence and risk of severe complications related to prematurity is reduced through the receipt of breast milk (Furman et al., 2003; Johnson et al., 2014). Premature infants who receive breast milk have lower rates of mortality and morbidity, especially caused by neonatal sepsis and necrotizing enterocolitis, compared to those who do not receive breast milk (Abrams et al., 2014; Cortez et al., 2018; Sisk et al., 2017). It is well-known and acknowledged that receipt of breast milk is as important (if not more so) for preterm infants compared to healthy-term infants.

Breast milk is a complex biological fluid with known benefits for maternal and infant health. The World Health Organization (WHO) and The American Academy of Pediatrics (AAP) recommend exclusively feeding human milk to infants during the first six months of life to achieve proper growth and development. After six months, breast milk should continue to be given along with nutritionally adequate and safe complementary food until the child is up to two years or beyond of age (Meek et al., 2022; World Health Organization, 2022). The AAP also suggests that all premature infants should receive breast milk or breast milk with pasteurized donor milk as the first choice rather than premature infant formula (Underwood, 2013). Numerous studies to date have confirmed the beneficial effects of feeding breast milk for the preterm infants in terms of host defense, neurological development and maturation, gastrointestinal development, reduction of late-onset sepsis, necrotizing enterocolitis, retinopathy of prematurity and chronic lung disease (Altobelli et al., 2020; Brown-Belfort, 2017; Kim et al., 2019; Muneer et al., 2018; Stepanovich & Donn, 2022). In addition, breast milk provides positive long-term effects on premature infant's health over infant formula. Compared to premature infants who receive formula milk, breastfed-premature infants have lower rates of metabolic syndrome, lower blood pressure, low-density lipoprotein levels, and less insulin and leptin resistance when they reach adolescence (Singhal et al., 2001, 2004).

Breast milk contains significant nutritional components that support premature infant growth and development. The macronutrient compositions of breast milk, a group of solid nutrients, have been shown to improve growth outcomes of preterm infant such as increased weight gain, length gain, and head and chest circumference (Belfort et al.,

2020; Lin et al., 2020; Rozé et al.,2012). Breast milk macronutrients also play crucial role in development of various organs in preterm infant (Mosca & Gianni, 2017). Several studies report positive correlation between breast milk macronutrient and premature infant's brain, respiratory, and immune system development (Boquien et al., 2018; Martin et al., 2016; Vohr et al., 2006). There are three essential macronutrients in breast milk, including carbohydrates, proteins, and fats. Carbohydrates are the primary macronutrients composition in breast milk. Generally, mature breast milk contains approximately 7% carbohydrates, with the majority being lactose (70%) and oligosaccharides (30%), respectively (Kim & Yi, 2020). Carbohydrates promote developing physiological function of microbiome in the intestine system, which supports digestion and strengthens the immune system in premature infants (Boudry et al.,2021). Carbohydrates in breast milk also improve the development of the central nervous system. Oligosaccharides have been shown to influence brain function in premature infants (Berger et al., 2020). Second, proteins are contained in the breast milk around 1% (Kim & Yi, 2020). Breast milk has a variety of proteins, including whey and casein, and various amino acids. Proteins in breast milk promote growth and cell function in the premature infant body (Amissah, et al., 2020). The essential protein amino acids in breast milk help build and regulate the preterm infant's immune protection (Ballard & Morrow, 2013). Third, fats are the second largest macronutrient, estimated at 3.8% in breast milk. The major component of fat in breast milk is triglycerides (around 95%) and it also includes many essential fatty acids, such as linoleic acid and alpha-linolenic acid (Kim & Yi, 2020). A high concentration of fatty acids in breast milk plays the most important role in the energy supply in preterm infants (nearly 50% of the total energy demand) and the developing brain and retina

(Muneer et al., 2018). Thus, the macronutrient concentrations in breast milk do not provide only a nutrition supply for preterm infants, but they offer the structural elements that positively affect premature growth and development.

Although breast milk is the optimal food for infants to produce and sustain growth and development, preterm infants have unique developmental needs and breast milk alone may not meet the recommended nutritional levels for optimal growth and development (Belfort et al., 2020). Since preterm infant have missed the last trimester placental transfer of nutrients, they may require higher nutrient intake than full-term infants in order to support their growth and development (Hay, 2013). NICUs need to use specialized fortifiers that are added to breast milk to optimize the nutrient intake of premature infants. Fortification of breast milk with energy, protein, and minerals is standard practice in many neonatal intensive units (NICU) around the world (Perrin et al., 2018). The purpose of these fortifiers is to fulfill the recommended nutrient levels when added to breast milk. However, researchers have found delayed postnatal growth among preterm infants who fed primarily fortified breast milk (Henriksen et al., 2009), suggesting that current fortification strategies may not meet nutrient requirements for all preterm infants. Because the exact composition of macronutrients is varied across mothers, it can be difficult to achieve consistent nutrient levels when adding fortifiers. It is possible that the variability of macronutrient contents in breast milk may contribute to growth deficits among preterm infants despite adding fortifiers. Therefore, understanding how factors may affect changes in breast milk macronutrients should be considered and further studied.

Breast milk macronutrients change dynamically and vary across individual mothers according to various factors. It has been reported that macronutrients in breast milk is affected by physiological, infant, and mother factors. (Azad et al., 2021; Bzikowska-Jura et al., 2020). Physiological factors such as stage of lactation and time of breast milk expression impact changing components of macronutrient in breast milk (Ballard & Morrow, 2013). The macronutrient content of breast milk can vary based on the stage of lactation. For example, colostrum, the milk produced in the first few days after birth, is higher in protein and lower in fat than mature breast milk. As lactation progresses, the composition of breast milk changes, with a decrease in protein and an increase in fat and carbohydrate content (Czosnykowska-Łukacka et al., 2018). In terms of time of breast milk expression, the breast milk expressed in the morning may have a higher carbohydrate and lower level of lipids content when compared to breast milk expressed in the evening (Moran-Lev et al., 2015). Changes in the macronutrients of breast milk are also influenced by infant factors, including gestational age and infant gender. For gestational age, previous studies show that breast milk macronutrients are variable between infants who are born prematurely and full-term infants. Premature-breast milk has more protein and higher levels of many bioactive molecules compared to term-breast milk (Belfort et al., 2020; Underwood, 2013). In addition, there is some evidence to suggest that the composition of breast milk may vary slightly depending on the infant sex. Studies derived from animal models have shown that breast milk produced for male infants has slightly higher fat and protein content than milk produced for female infants. (Quesnel et al., 2017; Robert & Braun, 2012). Moreover, maternal factors, including age, health condition, and maternal nutrition (BMI, and diet intake,) are

considered to have an impact on breast milk macronutrient composition (Bravi et al., 2016; Huang & Hu, 2020; Peng et al., 2021; Sims et al., 2020). Researchers have reported that older mothers may produce breast milk that is higher in carbohydrate and fat content than younger mothers (Lubetzky et al., 2015). Also, maternal health conditions, such as diabetes, hypertension, or obesity, can affect the levels of macronutrients in breast milk. Maternal nutritional status and diet have also been reported in their associations with macronutrients in breast milk. Maternal BMI can affect the quantity and quality of breast milk nutrients through multiple metabolic processes by changing in hormone levels (Novak & Innis, 2011). Mothers who have higher BMI may have higher levels of leptin, which can increase the production of fats in breast milk, resulting in higher level of breast milk fats in overweight mothers when compared to normal weight mother (Peng et al., 2021; Sims et al., 2020). However, the report of association between maternal diet and breast milk macronutrients in the existing literature are not consistent. Our recent scoping review show some studies reported significant effect of maternal carbohydrate and protein intake on higher level of proteins, fats and energy in breast milk (Hascoet et al., 2019; Huang & Hu, 2020; Hu et al., 2021; Zhang et al., 2021). In contrast, some studies in our review also reported no significant association between maternal diet and macronutrients content in breast milk (Aumeistere et al., 2019; Bravi et al., 2021; Bzikowska et al., 2018; Bzikowska-Jura et al., 2020; Rakicioglu et al., 2006; Titi et al., 2014). The varied results may be related to variability in methodological approaches and participants across studies. A broad range of difference using breast milk sampling methods and maternal nutrition assessment tools might affect breast milk concentration and its association with maternal nutrition. To resolve the current unclear knowledge

about this issue, it is necessary to utilize a standardized milk sampling procedure and validated maternal nutrition assessment in studies.

## **1.2 What is not known and in need of further study**

Breast milk macronutrient composition is highly variable across mothers during lactation. Physiological, infant, and mother factors are generally considered to be the influencing factors on dynamic and varied levels of macronutrients in breast milk. However, based on our scoping review, the effect of maternal nutrition factors, especially diet intake on breast milk macronutrients is not clearly defined. Studies have reported inconsistent results on this topic according to varied methodologies in regard to milk sample collection and maternal nutrition assessments. Similarly, researchers who have completed systematic reviews on breast milk macronutrient content have also reported that major challenges in comparing and understanding results on this topic include the lack of standardized collection procedures (e.g., time of day, type of milk removal, sequence of feeding/milk removal) and analysis protocols and tools, as well as a lack of standardized maternal nutrition assessments across studies (Adhikari et al., 2021; Bravi et al., 2016). As a result, a complete understanding of the influence of maternal nutrition factors on macronutrient composition in breast milk remains unclear. To advance our knowledge of maternal nutrition factors and their association with breast milk macronutrients, it is essential to apply standardized and validated milk sampling and maternal nutrition analytical methods to evaluate the relationship between breast milk macronutrients and maternal nutrition factors. Additionally, there is scarcity of research on the nutritional content of breast milk from Thailand. Little is known about the

influence of maternal nutrition factors (diet and nutrition status), and also other demographic mother and infant factors (such as age, education level, multiple pregnancies, mode of delivery, gestational age and infant sex) on the composition of milk from mothers giving birth prematurely in Thailand due to a lack of available data on this topic. Most published data are based on studies from western populations (Titi et al., 2014). As the cultural and environmental context can affect individual's nutrition, demographic data, and health, the existing data may not be applicable to Thai mothers. To address this issue, this study conducted to examine maternal nutrition factors, maternal demographic factors (maternal age, education level, multiple pregnancies, mode of delivery), and infant factors (gestational age and infant gender) and their association with breast milk macronutrients in Thai mother populations. Understanding of the impact of these variables on breast milk macronutrient composition provided information for developing a dietary and nutrition support program to improve breast milk nutrients for promoting appropriate growth and development in preterm infants. Also, the information could help identify mother-infant pairs who may have a higher risk of insufficient macronutrients in breast milk based on their nutrition and demographic factors.

### **1.3 Purpose of the study**

The primary purpose of this study is to identify factors affecting macronutrient levels in the breast milk from mothers of preterm infants in Thailand by examining maternal nutrition factors and other relevant factors. Additionally, the researcher examined the change in macronutrients in breast milk at different stages of lactation and the data of maternal nutrition during the first month of lactation as secondary outcome

measures. The lactation stages of this study were defined as the period of milk excretion during the first 1-2 weeks of lactation (colostrum & transitional milk) and 3-4 weeks of lactation (mature milk) (Christian et al., 2021). Aims and research questions guiding this study were:

**Aim 1.** To compare the breast milk macronutrient components at different stages of lactation (colostrum & transitional milk: 1-2 week of lactation) vs (mature milk: 3-4 week of lactation) and characterize the changes in macronutrients from colostrum & transitional to mature milk among Thai mother of preterm infants.

**Aim 2.** To describe maternal macronutrients intake (based on 24 hours food recall record), dietary habits (based on intake frequency of food products) and nutritional status during the first month of lactation among Thai mothers of preterm infants.

**Aim 3.** To explore how maternal nutrition factors (current diet intake of macronutrient, habitual diet based on frequency of food consumption, current BMI), maternal demographic factors (maternal age, education level, multiple pregnancy, mode of delivery), and infant factors (gestation age and gender) can affect macronutrient components in mature milk.

**Research question 1:**

Is there a difference in the level of macronutrient composition between the 1-2 week of lactation and 3-4 week of lactation among Thai mothers of preterm infants?

**Research question 2:**

What is macronutrient intake value, habitual diet intake, and nutritional status during the first month of lactation among mother Thai mother of preterm infants?

### **Research question 3:**

How maternal nutrition factors (current diet intake of macronutrient, habitual diet, current BMI), maternal demographic factors (maternal age, education level, multiple pregnancies, mode of delivery), and infant factors (length of gestation, and infant gender) can affect macronutrient components in mature milk?

### **1.4 Conceptual framework**

The conceptual framework guiding this study is based on the mother-breastmilk-infant triad concept developed by Bode and colleague (2020). This concept describes maternal physiology, breastmilk composition, and infant physiology as parts of a co-adapting system, with variations in each influencing the trajectory of infant development and maternal health. This concept also captures the study of breast milk that affects and is affected by interactions with both the mother and the infant. Bode et al. (2020) explains each component in the triad concept as follows:

The first concept, mother, refers to maternal physiology and behavior related to their breast milk composition and infant health. To better demystify the mother-breast milk- infant triad concept, it is critical to understand maternal factors and their influence on breast milk composition and infant health. Maternal nutrition is one of the most influential factors that affect lactation and infant growth and development. Three aspects of maternal nutrition could have impacted on breast milk composition and infant health outcomes, which are current dietary intake, nutrient stores in the body (e.g. BMI, body fat mass) and changing in nutrient due to hormones during lactation. For example, maternal consumption of foods rich in omega-3 fatty acids may increase the concentration of fatty

acids in breast milk, which can support infant's neurological and retinal development (Morrow & Dawodu, 2019). In addition, it is important to consider maternal demographic factors such as socioeconomic status as this facet may also affect maternal nutrition and consequently affect the composition of breast milk and infant health. Mothers of lower socioeconomic status and educational level may have limited access to healthy food, lack of knowledge about nutrition, poor environmental sanitation, and poor health behaviors such as smoking or substance use, which can lead to malnutrition during lactation (Ravaoarisoa et al., 2018). Also, maternal age less than 20 years (teenage mothers) have been reported as a factor that influences the occurrence of malnutrition in mothers. Because teenage mothers may be more likely to have unplanned pregnancy and come from low-income families, they have a higher risk of malnutrition during lactation (Ramadhani et al., 2021). Poor nutrition in mothers associated with socioeconomic status can impact breast milk composition during lactation. Malnourished mothers due to lower of socioeconomic level may produce breast milk with lower levels of fat, which can affect the growth and development of the infant (Leghi et al., 2020).

Breast milk in the triad concept refers to biological fluid from mothers to infants that contains macronutrients, micronutrients, bioactive (non-nutrition components such as immunoglobulin, hormone, protein, HMOs, cells, Peptides, Cytokines, mRNA), and microbiome. There are three sources of breast milk composition: some are synthesized in the mammary secretory cell from precursors in the plasma, some are produced by other cells in the mammary gland, and others are transferred directly from plasma to milk. Breast milk composition, including these macronutrients, micronutrients, bioactive, and microbiome, is dynamic and varies for many different factors previously discussed in this

chapter (e.g. stage of lactation, time of milk expression, length of gestation,) both in the same individual and between mothers.

Finally, infant refers to health and wellbeing among infants who receive breast milk from their mother. Infant health is associated with the breast milk composition and mothers. Both positive and negative health outcomes in infants are influenced by breast milk composition and mother nutrition. There has been research reported that well-nourished mothers have increased levels of nutrients in their breast milk composition when compared to malnourished mothers (Mane et al., 2018). As a result, infants of well-nourished mothers may have a greater weight and length gain than those in malnourished mothers. Because breastfed infants are entirely dependent upon the mother for nutrition, the alteration of maternal nutrition and breast milk composition can impact infant health. In addition to maternal factors influencing breast milk composition, we are beginning to understand that infant factors also influence breast milk composition. For instance, the gestational age of an infant may influence the macronutrient composition of breast milk. Breast milk from mothers of premature infants have higher protein concentrations than mothers of term infants (Underwood, 2013). Because premature infants have increased protein needs for growth and development, premature breast milk needs to have higher protein content to support nutritional need (Léké et al., 2019).

For this study, the mother-breast milk- infant triad concept was applied to explore the association of maternal nutrition factors, mother demographic factors, infant factors, and macronutrient concentration in breast milk. First, maternal nutrition factors including current dietary intake of macronutrients, habitual diet intake, and BMI were used as explanatory factors for exploring association with breast milk macronutrients. Since other

demographics of mother and infant factors may have an impact on breast milk composition based on the concept as described above, selected demographic of mother and infant factors, including maternal age, education level, multiple pregnancies, mode of delivery, length of gestation, and infant sex were also taken into account as explanatory factors. These variables were collected from the medical chart after the mother has a premature birth. Next, macronutrient levels in mature breast milk were determined as the dependent factor. Breast milk samples were collected from a specific time to avoid affecting the time of milk expression on breast milk composition. Finally, the connection of maternal nutrition factors, maternal demographic factors, and infant factors and macronutrient concentration in mature breast milk were examined.

## **1.5 Definition of variables**

This study includes eight independent variables and one dependent variable as follows:

### **1.5.1 Independent variables**

**1.5.1.1 Maternal dietary intake** in this study is defined as the consumption of a nutrient or food in mothers during the first month of lactation period. The study evaluated maternal diet intake using two different methods. The first method is based on the mother's current dietary intake of macronutrient, which was assessed using non-consecutive 24-hours dietary food recall record for one weekday and one weekend day. The second method is based on the mother's dietary habits over the past month, which was assessed using food frequency questionnaires (FFQs).

**1.5.1.2 Maternal body mass index (BMI)** in this study refers to basic anthropometric measures of body weight and height of mothers during the first month of lactation period. Maternal body weight and height was measured following standard techniques using a standing digital scale and portable stadiometer. Then BMI was determined by calculating the standard equation: weight/height (kg/m<sup>2</sup>).

**1.5.1.3 Maternal age** refers to the age of a mother at the time of giving birth to preterm infants.

**1.5.1.4 Educational level** refers to the highest level of education completed by the mother.

**1.5.1.5 Multiple pregnancies** refer to the mothers who has recently given birth two or more infants.

**1.5.1.6 Mode of delivery** refers to the method by which preterm infant is delivered from the mother's uterus to the outside world. There are two modes of delivery in this study, including Vaginal delivery and Cesarean delivery

**1.5.1.7 Gestational age** at birth refers to the number of weeks of pregnancy at the time of delivery. Gestational age is typically calculated based on the date of the mother's last menstrual period and confirmed by ultrasound measurements of fetal size and growth. This study included gestational age of preterm infant between 30-36 weeks.

**1.5.1.8 Infant sex** refers to the biological classification of preterm infant as male or female based on their genital anatomy.

## 1.5.2 Dependent variable

**1.5.2.1 Breast milk macronutrient composition** in this study is explained as the solid nutrients in breast milk, including carbohydrates, protein, fat, and energy (Kim & Yi, 2020). The breast milk of this study was obtained from the entire one breast expression in the morning after breakfast and before lunch (7.00 AM – 11 PM). This study collected breast milk samples at two different time points because the composition of breast milk changes over time due to the stage of lactation. The first sample was collected during the first 1-2 weeks after birth when the mother is producing colostrum and transitional milk, and the second sample was collected during weeks 3-4 when the mother is producing mature milk. However, only the macronutrient composition of the mature milk sample was examined as the dependent variable to explore their association with the independent variables. This is because mature milk is the primary source of nutrition for infants and is produced for over extended period compared to colostrum & transitional milk, which is only produced in the first few days after birth. To measure macronutrient composition in mature milk, MIRIS Human Milk Analyzer was conducted in this study. The carbohydrate, protein, and fat were measured in grams (g) per 100 ml of milk, and energy levels was measured in kcal per milk 100 ml. The carbohydrate levels include the content of 70% lactose and 30% oligosaccharides of breast milk. The protein levels refer to a true protein which represents only actual protein source of nitrogen in breast milk. The fat levels refer to breast milk triglycerides, diglycerides, free fatty acids, phospholipids, and cholesterol (Miris, 2020).

## 1.6 Summary

Breast milk is a complex biological fluid with nutritive and other bioactive factors that are incredibly important for preterm infant health and development. It is known that macronutrient composition in breast milk varies over the lactation period between and within mothers of preterm infants. One challenge with the differences in breast milk macronutrients is that it can lead to inadequate nutrients consumed in preterm infants even after fortification in breast milk. As a result, it can lead to insufficient growth and development among preterm infants. There is a need for studies to examine breast milk macronutrients and their associated factors. Various factors, including physiology, infant, and mother, have been reported that affect breast milk macronutrients (Azad et al., 2021; Bzikowska-Jura et al., 2020). However, studies that had directly explored the impact of a mother's diet on the variability of breast milk macronutrients remain unclear.

Additionally, there has been a lack of research in Thailand investigating the relationship between the macronutrient content in breast milk and demographic factors of both the mother and infant, such as age and education level etc. Based on the gap in this phenomenon, the purpose of this study is to explore the influence of maternal nutrition factors during the lactation period (diet intake and BMI), maternal demographic factors (maternal age, education level, multiple pregnancies, mode of delivery), and infant factors (length of gestation and infant gender) on macronutrient composition in breast milk among Thai mothers of premature infants. For the next chapter, scoping review about maternal nutrition factors and breast milk nutrient composition was presented. It provides essential information for this dissertation by providing how maternal nutrition factors may relate to macronutrient in breast milk.

## CHAPTER 2

### LITERATURE REVIEW

#### **The Influence of Maternal Nutrition on Breast Milk Macro and Micronutrient Composition: A Scoping Review**

##### **Abstract**

**Objective:** To explore the influence of maternal nutrition factors, including dietary intake, nutritional status (BMI), and nutritional supplementation during the lactation period, on macro and micronutrient composition in breast milk.

**Data Sources:** The electronic database of PubMed, Scopus, and Web of Science were used to identify original studies published in English that presented findings on the relationship between maternal nutritional factors and macro and/or micronutrient composition in breast milk.

**Study Selection:** Using inclusion and exclusion criteria, 4,482 studies were initially assessed through title and abstract review. Further screen resulted in full review of 65 studies. Checking references lists added 3 additional studies. Finally, in-depth review of these 68 articles resulted in a final sample of 22 studies.

**Data extraction:** Studies were reviewed for information related to maternal nutrition factors and the association with macro and micronutrients in breast milk.

**Data Synthesis:** Most studies found maternal BMI and supplement consumption have an effect on breast milk composition of macro and micronutrients, while there are inconsistent results on the relationship between maternal diet and breast milk nutrients. Additionally, methodologies varied substantially across studies, particularly for milk sample collection, milk analysis methods, and maternal nutrition assessment.

**Conclusion:** Maternal nutrition factors, especially BMI and supplement use are associated with breast milk nutrients. However, the sampling and analysis methodology on this topic is varied and inconsistent. Standardized procedures for milk sample and analysis and validated maternal nutrition assessments should be implemented to further investigate the impact of maternal nutrition factors on macro and micronutrients in breast milk.

**Keywords:** Maternal diet, maternal BMI, breast milk composition, macronutrient, micronutrient

**Precis statement:** Maternal nutrition factors, especially BMI and supplement use are associated with breast milk nutrients.

**Callouts:**

-There is diverse available information on the influence of maternal nutrition intake, BMI and supplementation on breast milk macro and micronutrients. (Introduction)

-Maternal diet intake, BMI, and supplementation have a potential impact on the macro and micronutrient composition of breast milk. (Result)

-Future research is needed on the use of well-defined and standard parameters to examine maternal nutrition factors and their association with macro/micronutrients in breast milk. (Conclusion)

## 2.1 Introduction

Breast milk is widely accepted as the optimal nutrition for infants during early life. The World Health Organization (WHO) and the American Academy of Pediatrics (AAP), suggest that breast milk is a critical nutrition source for all infants in the first sixth months of life and along with suitable complementary food up to two years of age or beyond (Meek et al., 2022; World Health Organization, 2021). Breast milk contains vital macro and micronutrients essential to ensure infants' healthy growth and development. Macronutrients of breast milk, including lipids, proteins, and carbohydrates, represent the primary source to supply energy for infants and provide critical nutrients, which are needed as structural elements with a positive effect on infant growth and body composition (Mosca & Gianni, 2017). Other micronutrients of breast milk, such as vitamins and minerals, are fundamental for the neurocognitive development of infants (Lockyer et al., 2021). However, the composition of breast milk macro and micronutrients changes dynamically depending on various factors. Thus, it is crucial to understand the nutritional composition of breast milk and the factors relating to its composition to promote infant health and development.

Maternal nutrition factors are considered to have an effect on the nutritional composition of breast milk. Dietary intake and nutritional status (Body Mass Index – BMI) have been addressed as maternal nutrition factors that could impact breast milk macro and micronutrient composition (Azad et al., 2021). Maternal diet and nutrition status can influence the quantity and quality of milk composition through multiple metabolic processes (Novak & Innis, 2011). In the comparison study of breast milk nutrients between well-nourished and undernourished women from India, there has been

a report of higher macronutrient and micronutrient concentrations such as protein and copper in the breast milk of well-nourished than malnourished women (Mane et al., 2018). Fatty acids in breast milk have been reported as associated with maternal nutrition status at the time of lactation. Maternal undernutrition has presented low concentration of breast milk fat concentration (Leghi et al., 2020). Alterations in maternal diet and nutrition status that change the composition of breast milk macro and micronutrients play an essential role in infant health and development since breast milk nutrients serve as the nutritional standard for infants. However, not all breast milk macro and micronutrients are impacted by variations in maternal nutrition factors. The concentrations of carbohydrates in breast milk are similar among women, and there is no association with maternal diet factors (Adhikari et al., 2021). It has remained unclear the effect of maternal diet and nutrition status on all aspects of breast milk composition and the extent of any influence on infant growth. Also, little is known about the optimal breast milk nutrient composition level depending on maternal nutrition factors. Thus, we still need further studies to examine the association of maternal diet and nutrition status with macro and micronutrient concentration in breast milk.

In addition, the use of dietary supplements during the lactation period, particularly fatty acids, fat-soluble vitamins, vitamin B and C, may impact micronutrient levels in breast milk (Keikha et al., 2017). The maternal diet, especially micronutrient intake, is not always optimal. Most lactating women are recommended to use a dietary supplement that contains micronutrients and vitamins to support breast milk micronutrient concentrations (Ballard & Morrow, 2013). Adequate consumption of micronutrients is vitally important during lactation as they are necessary for the proper growth and

development of the infant. For example, maternal supplementation with long-chain polyunsaturated fatty acid, including omega-3, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA), has a positive association with breast milk fatty acid levels that provide a fundamental source for infant visual, motor, and cognitive development (Bernardi et al., 2012).

However, there is limited synthesis of literature on the effect of maternal nutrition factors on macro and micronutrients of breast milk. Previous narrative reviews have mainly focused on one maternal nutrition factor referring to maternal dietary intake and a selected breast milk component, particularly fatty acids (Bravi et al., 2016; Keikha et al., 2017). Limited attention has been paid to the effect of maternal nutrition statuses such as BMI and supplementary nutrition during lactation on breast milk macro and micronutrient composition. Therefore, this scoping review aimed to summarize the existing evidence on the effects of three maternal factors: maternal dietary intake, nutritional status (BMI), and supplementation use during the lactation period on macro and micronutrient composition in breast milk. The question guiding this review is therefore, “what is known about the influence of maternal dietary intake, BMI, and supplementation on macro and micronutrient composition in breast milk?”

## **2.2 Methods**

### **2.2.1 Eligible criteria**

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines extension (PRISMA-ScR) for guiding our scoping review (Tricco et al., 2018).

We selected primary research examining the association between macro and/or micronutrient composition in breast milk related to at least one nutrition-related maternal factor, such as dietary intake, BMI, and supplementation in our review. We included studies only the English language and publication from 1990-to 2022. We excluded studies if they (1) focused on animals or laboratory designs; (2) did not clearly explain the methodology used for determining the composition of breast milk; (3) included only colostrum or transitional breast milk instead of mature milk; (4) focused on maternal dietary, BMI, or supplementation only during pregnancy rather than lactation period; (5) were published as a book chapter, letter, and/or review publication.

### **2.2.2 Information sources and Search Strategy**

We identified studies by searching electronic databases and scanning reference lists of articles included in the review. We applied our search strategy to PubMed, Scopus, and Web of Science for articles published from 1990 to March 2022. The search strategy included a combination of terms with Boolean operators as follows: (“maternal diet” OR “maternal nutrition” OR “maternal eating”) AND (“Human milk” OR “breast milk” OR “breast milk composition” OR “human milk composition”). We completed our searching between February and March of 2022.

### **2.2.3 Selection of sources of evidence**

We used the EndNote program for managing and handling extracted references that were searched from multiple databases. First, we removed duplicate articles with the EndNote function before importing all articles into the Rayyan software application, which is a tool designed to help screening titles and abstracts in the review process (Ouzzani et al., 2016). We also manually checked for duplicate articles that might have

been missed. Then, we used Rayyan to screen article titles and abstracts. Based on the manual Rayyan screening, we included relevant paper (n=65) for full-text review. To increase sensitivity and obtain more related studies, we checked the reference lists of the included articles, resulting in 3 additional papers for full-text review. We used the PRISMA flow diagram (see Figure 1) to show the search selection process. This review included a final sample of 22 articles.

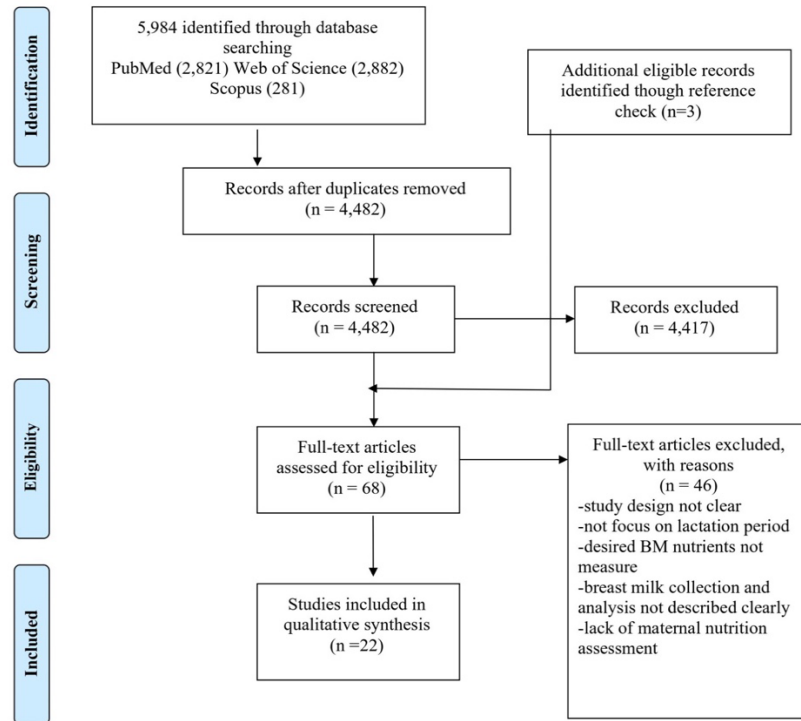


Figure 1: PRISMA diagram of scoping review methods

#### **2.2.4 Data charting and items**

We extracted data from the articles included in the full-text review using an extraction form developed by the first author. Data extracted consisted of year of publication, geographical area, study design and aims, characteristics of participants, milk sample collection and analysis, maternal nutrition assessment (dietary assessment, anthropometric measurement used to examine maternal nutrition status, maternal supplemental nutrition), and significant findings of the study.

### **2.3 Result**

#### **2.3.1 Descriptive Characteristics of identified studies and sample**

Of twenty-two studies included in this scoping review, nineteen were observational, and three were experimental studies. Studies contained in this review were published from 1990 to 2022, of which most studies were published after 2011 (82%). The majority of studies were conducted among European (55%) and Asian women (35%), followed by studies among Ethiopians (5%) and North Americans (5%). The age of women included in the studies ranged from 20 to 44 years, and most of the participants were women with full-term infants. While only one study was conducted in women who had preterm infants. The sample size varied between studies, ranging from 10 to 436, and the lactation duration in 91% (n=20) of the studies ranged between 1 and 12 months. In only one study, milk was included up to 21 months postpartum. (Aumeistere et al., 2019).

### **2.3.2 Breast milk collection and analysis**

Breast milk collection procedures varied across studies. Most studies (n=14) collected breast milk samples at multiple time points during lactation and milk sampling was done in the morning (Aumeistere et al., 2019; Bravi et al., 2021; Daniels et al., 2019; Fidler et al., 2000; Gibson et al., 2020; Huang & Hu, 2020; Hu et al., 2021; Makela et al., 2013; Mazurier et al., 2017; Nakamori et al., 2009; Rakicioglu et al., 2006; Sims et al., 2020; Titi et al., 2014; Zhang et al., 2021). Five studies used milk samples collected from 24-hour milk sampling. One study analyzed pooled milk from various days, and the rest of the studies (n=2) did not provide clear information on time of milk collection. Most studies used a combination of hand expression and electric/battery breast pumps to collect milk samples. However, four studies did not report method of breast milk expression (Bzikowska-Jura et al., 2019; Hascoet et al., 2019; Mazurier et al., 2017; Peng et al., 2021). Milk sample volumes collected varied, ranging from 1 to 500 ml and mostly obtained at pre-and-post infant feeding. Most studies specified that breast milk was stored at a freezing temperature ranging between -20°C to -80°C. However, four studies did not report breast milk storage temperature before analysis (Aumeistere et al., 2019; Bzikowska et al., 2018a; Bzikowska-Jura et al., 2021; Huang & Hu, 2020). For the breast milk analysis, a variety of methods were used. Most of the studies (n=13) used MIRIS human milk analyzer for macronutrient breast milk analysis. Micronutrient breast milk analysis was used by various methods, including Kjeldahl, Rose-Gott-Lieb, chromatographic, Bligh-Dyer, ICP-MS, Agilent technologies, and Shimadzu AA-600 model (Argaw et al., 2021; Bzikowska-Jura et al., 2019; Daniels et al., 2019; Fidler et al.,

2000; Gibson et al., 2020; Makela et al., 2013; Mazurier et al., 2017; Nakamori et al., 2009; Peng et al., 2021; Rakicioglu et al., 2006).

### **2.3.3 Maternal nutritional assessment**

Across the identified studies, the most frequently used (n=13) dietary intake assessment method was the self-reported food record for 24 hours and 3 days. Two studies used personalized interviews with dietitians (Bravi et al., 2021; Hascoet et al., 2019). Another method used was the food frequency questionnaire (Aumeistere et al., 2019; Bravi et al., 2021; Bzikowska-Jura et al., 2021; Hascoet et al., 2019; Zhang et al., 2022). Four studies classified maternal diet pattern by using component factor analysis to determine nutrients in the maternal diet (Bravi et al., 2021; Huang & Hu, 2020; Hu et al., 2021; Zhang et al., 2021). Six studies calculated the nutrient content of maternal diet compared with dietary reference intakes from their countries (Aumeistere et al., 2019; Bravi et al., 2021; Gibson et al., 2020; Hu et al., 2021; Nakamori et al., 2009; Titi et al., 2014). Five studies estimated maternal nutrient intake by computer software (Bzikowska et al., 2018b; Bzikowska-Jura et al., 2019; Bzikowska-Jura et al., 2021; Hascoet et al., 2019; Rakicioglu et al., 2006). For maternal BMI, basic anthropometric measures of height and body weight were assessed for determining maternal BMI. BMI was calculated and classified using the standard equation:  $\text{weight/height (kg/m}^2\text{)}$ . Three studies categorized women into two groups according to their BMI: normal weight and overweight (Makela et al., 2013; Peng et al., 2021; Sims et al., 2020) and two studies evaluated body composition using the Maltron BioScan 920-II (Bzikowska et al., 2018b; Bzikowska-Jura et al., 2020; Bzikowska-Jura et al., 2021). Pre-pregnancy BMI and BMI during lactation were assessed for all selected studies.

### 2.3.4 Main results

Hereafter, we explain the results concerning the influence of maternal nutrition on breast milk macro/micronutrients reported in the identified studies, ordered by maternal diet, maternal BMI, and maternal supplementation (see table 1).

**The effects of maternal diet on macronutrient composition in breast milk.** A total of ten studies investigated maternal dietary intake and its association with macronutrient composition in breast milk. Of these, six studies reported no significant association between maternal dietary intake and macronutrients in breast milk, such as fats, proteins, and lactose content (Aumeistere et al., 2019; Bravi et al., 2021; Bzikowska et al., 2018b; Bzikowska-Jura et al., 2020; Rakicioglu et al., 2006; Titi et al., 2014). Aumeistere et al. (2019) examined the effect of dietary macronutrient intake on breast milk composition among lactating women in Latvia. The study found no significant correlation between maternal intake of macronutrients and proteins, fats, and lactose composition in breast milk. Similarly, studies conducted in Italy, Poland, Turkey and China showed no relationship between maternal intake of any dietary nutrient and the macronutrient composition of breast milk (Bravi et al., 2021; Bzikowska et al., 2018b; Bzikowska-Jura et al., 2020; Rakicioglu et al., 2006; Titi et al., 2014). In contrast, four studies reported significant association between maternal dietary intake and breast milk macronutrients (Hascoet et al., 2019; Huang & Hu, 2020; Hu et al., 2021; Zhang et al., 2021). A study conducted in France showed a significant positive relationship between milk protein, fat, and calorie levels and maternal carbohydrate intake (Hascoet et al., 2019). Likewise, a study by Zhang et al. (2021) reported a positive relationship between maternal protein intake and total proteins in breast milk. Two studies from China

also found that maternal dietary patterns were associated with macronutrients in breast milk. Diets classified as “High-in-animal-foods” were positively associated with carbohydrates in breast milk (Hu et al., 2021 pp.). In addition, dietary patterns with high intake of protein were correlated to higher protein and energy contents in breast milk (Huang & Hu, 2020).

#### **The effects of maternal diet on micronutrient composition in breast milk.**

Seven studies explored the association of maternal dietary intake with breast milk micronutrients. The majority of studies (n=6) reported associations between maternal micronutrient intake and breast milk micronutrient concentration (Bzikowska-Jura et al., 2019; Bzikowska-Jura et al., 2021; Daniels et al., 2019; Gibson et al., 2020; Rakicioglu et al., 2006; Zhang et al., 2021). A study conducted in Poland reported a significant positive relationship between maternal zinc and iron intake, and zinc and iron concentrations in breast milk (Bzikowska-Jura et al., 2021). However, another study by Nakamori et al. (2009) showed the opposite results. There was no significant association between maternal micronutrient intake and breast milk micronutrient concentrations of iron, zinc, and copper. Overall, the studies assessing maternal dietary intake and association with macro or micronutrient content in breast milk present inconsistent results.

**The effect of maternal BMI on macronutrient composition in breast milk.** Six studies in this review explored the potential association between maternal BMI and macronutrient composition in breast milk. All studies found that maternal BMI significantly influences the macronutrient composition of breast milk (Bzikowska et al., 2018a; Bzikowska-Jura et al., 2018b; Bzikowska-Jura et al., 2020; Peng et al., 2021; Sims et al., 2020; Titi et al., 2014). Four of these studies were observational and reported a

significant positive association between pre-pregnancy maternal BMI, current maternal BMI during lactation, maternal fat mass and total proteins, fats, and energy of breast milk content (Bzikowska et al., 2018a; Bzikowska-Jura et al., 2018b; Bzikowska -Jura et al., 2020; Titi et al., 2014). Two studies classified women depending on their BMI: normal weight and overweight. Women who were overweight presented higher total energy, fat, and protein in breast milk when compared to normal weight (Peng et al., 2021; Sims et al., 2020).

#### **The effect of maternal BMI on micronutrient composition in breast milk.**

Two studies examined the relationship between maternal BMI and micronutrients of breast milk (Bzikowska-Jura et al., 2021; Makela et al., 2013). Both studies found that higher maternal BMI was correlated to higher micronutrient levels in breast milk. The study from Poland found that higher maternal body fat mass was significantly related to higher zinc concentration in breast milk (Bzikowska-Jura et al., 2021). This was consistent with the study from Finland which found that higher maternal BMI was also associated with higher amounts of saturated fatty acids in breast milk (Makela et al., 2013).

**The effect of maternal supplementary nutrition on macro and micronutrient composition in breast milk.** Three experimental studies included in this review evaluated the association between maternal supplementary nutrition and breast milk micronutrients. All studies supported a positive association of maternal supplement and its reflection in fatty acid composition of breast milk (Argaw et al., 2021; Fidler et al., 2000; Mazurier et al., 2017). Argaw et al. (2021) evaluated the efficacy of fish-oil (FO) supplementation of lactating women on breast milk DHA and EPA concentrations. The

study found that women who received fish-oil during lactation had a significantly higher concentration of breast milk DHA and EPA concentration than those who received corn oil capsules ( $p < 0.001$ ). The findings are consistent with the study from Germany where women who received two capsules, containing 200 mg of DHA/day, had significantly higher DHA concentrations in breast milk than those who received placebo oil without DHA component ( $p < 0.003$ ) (Fidler et al., 2000). Similarly, in the study conducted by Mazurier et al. (2017), women were randomized to one of four groups: olive oil group (O group, control), an omega-3–enriched margarine group (M group), a rape-seed oil group (R group), and an omega-3–enriched margarine plus rapeseed oil group (MR group). The fatty acid profile in breast milk was significantly higher in women who received omega 3 supplement (group MR, R, and M) when compared to non-omega 3 supplement (group O) ( $p < 0.003$ ). Evidence is limited in this review regarding the relationship between maternal supplementary nutrition and macronutrient composition in breast milk.

## **2.4 Discussion**

In this scoping review, we identified 22 original studies addressing the association between maternal nutrition factors, including maternal dietary intake, BMI, and supplementation, and macro and micronutrients in breast milk. Evidence appears that maternal BMI and supplementary nutrition may affect macro and micronutrient composition in breast milk. However, the review identified inconsistent results about the influence of maternal dietary intake on macro and micronutrients in breast milk. The diversified results may relate to variability in sample size, milk sample collection and analysis methods, and maternal dietary assessments. In term of sample sizes, the number

of women in studies included in this review ranged from 10 to 436. Six of studies were conducted among less than 50 participants. Small sample size may limit statistical power to identify relationship between maternal diet and breast milk composition. Moreover, there is different milk sample collection and analysis methods, which could impact the breast milk compositions reported and their association with maternal diet. 24-hour breast milk collection is considered the most representative method to collect milk samples to account for circadian variation effect. However, only five studies conducted a 24-hour breast milk collection, which may affect their relationship with maternal diet. Likewise, methods used to measure macro and micronutrient content varied across studies. Different milk analysis methods, including gas chromatography-mass spectrometry (GC-MS), ultraperformance liquid chromatography (UPLC), Bligh–Dyer, Gerber, Kjeldahl method, ICP-MS, Agilent technologies, YSI model 2700, Roesse–Gottlieb, and Shimadzu AA-600 model can impact the breast milk composition reported. Furthermore, a diverse set of tools to evaluate maternal diet intake ranging from estimated food record for 1 or more days to food frequency questionnaires were shown in the review. The variability in maternal dietary intake assessments can influence the actual evaluation of mothers' nutrient intake and its association with breast milk composition. Thus, further studies on the association between maternal dietary intake and breast milk composition should conduct optimal standardized approaches for milk collection and analysis, a clear definition of maternal dietary intake which will help to select the most suitable assessment tools, and a combination of dietary measurements that may control the potential measurement error.

Additionally, beyond different methodological issues, many studies did not account for potentially relevant confounding factors, including gestational age, stage of lactation, and postpartum timing. Insufficiently accounting for these factors increases variation in macro and micronutrient composition in breast milk and maternal nutrition, which may attenuate the association between breast milk nutrients and maternal dietary intake under study. For example, breast milk composition from women who give birth prematurely differs from that of women who have full-term births. Preterm milk is higher in protein, fat, free amino acid, and sodium than term milk during the first few days after birth. Then, over the first few weeks following birth these breast milk nutrient levels decrease similar to term milk composition (Gidrewicz & Fenton, 2014; Underwood, 2013). In this review, most studies collected milk samples from women who had full-term births. There was only one study where milk was collected from women who gave birth prematurely. Also, one study did not clearly report gestational age. So, we still need a clear selection of women and infants under the same condition such as gestational age to control error result. Also, different stages of lactation may impact macro/micronutrient levels in breast milk. Colostrum milk, produced by mothers during the first few days after delivery, contains a higher concentration of proteins and lipids and a lower concentration of lactose when compared to mature milk, the final milk that is produced occurring after two weeks postpartum (Ballard & Morrow, 2013; Czosnykowska-Łukacka et al., 2018). Most of the studies collected milk sample from women at least one month postpartum that represent mature milk sampling. Few studies (n=2) started collecting milk from some women within their sample at the first week postpartum, which might have included some colostrum and transitional milk. Moreover, postpartum timing can affect breast milk

composition. Five studies collected milk samples more than 6 months postpartum. Prolonged lactation especially more than 6 months can impact the variation in breast milk nutrients. The macronutrient content of breast milk decreases significantly around 12 months postpartum when compared with milk expressed by women in the first 6 months (Czosnykowska-Łukacka et al., 2018). Further studies on the role of maternal diet on breast milk composition should account for differences in stage of lactation and postpartum timing to create a more rigorous methodological design.

## **2.5 Implications for nursing**

The nutritional components in breast milk are significant in promoting infant growth and development. This scoping review shows evidence that maternal nutrition factors, including dietary intake, BMI, and supplement use, may play a role in influencing breast milk nutrient concentration. Taking care of women's health, in terms of diet and nutritional status, during the lactation period could represent a strategy to promote infant health. Nurses who care for women in the postpartum period should focus on monitoring women's diet, BMI, and supplementation because it is beneficial in improving breast milk nutrients and may affect the infant's health. The outcomes of this review can help inform nutritional guidelines for lactating women who need to increase micronutrient content in their breast milk.

## **2.6 Limitations**

A potential limitation to this scoping review is selecting only original journal articles published in English that may cause selection bias. Further, the review examines

only a limited number of available scientific papers. Despite these limitations, this review is strengthened by its compliance to the PRISMA-ScR guidelines and robust search strategy conducted in multiple databases.

## **2.7 Conclusion**

This scoping review provides an overview of the studies that reported maternal dietary intake, maternal BMI, and supplementary nutrition and their association with breast milk composition of macro and micronutrients. The outcomes of this review also highlight the diversified methodological approach in this topic such as breast milk collection and analysis, and maternal nutritional assessments. Future studies should clearly define maternal nutrition parameters and follow standardized milk collection, storage and analysis procedures, as well as utilize robust maternal nutritional assessment tools. In addition, controlling confounding factors is important to ensure strong, reliable findings in future studies.

Table 1 Summary of finding of scoping review

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
<b>Observational Studies investigating maternal diet/maternal BMI and macro/micronutrient composition in breast milk</b>					
Aumeistere et al. 2019 Latvia.	To examine the effect of dietary macronutrient intake on human milk composition	- 61 women (gestational age at birth not reported) with median age 31 years at postpartum age of 1-21 months	60 ml of hind milk was collected from multiple pumpings after infant feedings once during: morning, midday, evening by hand expression, breast pump, or a combination of both methods; samples were frozen before analysis (no temperature specified) and various methods were used for	<b>Dietary intake:</b> using 3 days of diet recorded and food frequency questionnaire (FFQ); calculating maternal macronutrient intake by comparing with Finnish food	<b>-no significant correlation</b> between maternal macronutrient intake and protein, fat, and lactose composition in BM (p > 0.05).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
			macronutrients BM analysis, including protein content using Kjeldahl method, fat content using Gerber method and lactose using chromatography method.		
Bravi et al.  2021  Italy	To examine the association between maternal dietary patterns and BM	300 women giving birth to term infants with age range 25-41 years at	30-50 ml of breast milk by hand expression or breast pump was collected in the morning after breakfast and before lunch; samples were stored at -70°C; BM macronutrient analyzed by	<b>-Dietary Intake:</b>  FFQ was collected by dietician interview; maternal dietary pattern was identified by factor analysis	<b>-No association</b>  between maternal diet and macronutrient in BM.

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	macronutrient and fatty acid composition	postpartum age of 6 weeks	Miris HMA™; FA analyzed by gas chromatography		-Only BM fatty acids have a weak positive correlation with maternal diet pattern (p< 0.05).
Bzikowska et al. 2018a Poland	To investigate associations between human milk composition and maternal	40 women giving birth to term infants with median age 29.5 years at first month of lactation	5-10 ml of breast milk by pump or hand expression was collected at pre and post feeding within 24 hours (4 time/day); no report of type of storage before analysis; BM	<b>BMI measurement:</b> weight and height during pre-pregnancy and post-pregnancy were recorded to calculate BMI	- Pre-pregnancy BMI and BMI during lactation were <b>positively related</b> to lipid, dry matter and

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	nutritional status (BMI)	(collected at 3-4 weeks postpartum)	Macronutrients analyzed by Miris HMA™		energy breast milk content (p<0.05)
Bzikowska-Jura et al. 2018b Poland	To investigate the association of maternal diet and nutrition status with breast milk composition.	40 women giving birth to term infants with mean age 31.1 years at postpartum age of 1-6 months	5-10 ml of breast milk by breast pump or hand expression was collected at pre and post feeding over 24-hours at 4 time points; samples were collected at 1,3, 6 months PP and stored - 20°C until analysis; BM Macronutrients analyzed by Miris HMA™.	<b>Dietary Intake:</b> using self-report 3-day dietary record; calculating nutrient diet by Dieta 6.0 nutritional software  <b>BMI measurement:</b> Body weight and height were measure at	<b>-No significant relationship</b> between breast milk composition and maternal diet.  -Maternal fat mass, protein mass, and BMI were positively

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
				1,3, 6 months; body composition assessed using the Maltron BioScan 920-II analyzer.	associated with BM protein and fat content ( $p < 0.05$ ).
Bzikowska-Jura et al. 2019 Poland	To examine the association between breast milk fatty acid levels and maternal current dietary intake	32 women giving birth to term infants with mean age of 30.9 years at postpartum	5-10ml of breast milk was taken at pre and post feeding over 24 hours at 4 time points; no report milk expression method; samples were stored in the refrigerator (-4 °C) during 24 hours study period and then at	<b>Dietary Intake:</b> using 3-day dietary record for maternal current dietary intake; FFQ was collected for maternal habitual dietary intake (recall in	-There was <b>correlation between maternal habitual intake of fatty food products (fatty</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	and habitual dietary intake.	age of 2 to 4 weeks	-20°C for analysis; BM lipid analyzed by Miris HMA™; BM fatty acid assessed by gas chromatography	the last 3 months); calculating maternal fatty acids intake by Dieta 5.0 nutritional software.	<b>fish) and BM fatty acid levels</b> (P <0.05).
Bzikowska-Jura et al. 2020 Poland	To investigate the influence of maternal diet and BMI on macronutrient composition of	-n=77 women giving birth to term infants with average age 32.4 years at postpartum	5-10 ml of mature milk by pump or hand expression was obtained at pre and post feeding over 24 hours at 4 time points; samples were stored in the refrigerator during the 24 hours study period and then at -20°C	<b>Dietary intake:</b> using 3 days dietary records <b>BMI measurement:</b> Weight and height at pre and post pregnancy were recorded to calculate BMI; body	- There was <b>no association</b> between maternal diet intake and BM composition -Pre-pregnancy BMI, current BMI,

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	mature human milk	age of 4 to 8 weeks	until analysis; BM Macronutrients analyzed by Miris HMA™	composition assessed using the Maltron BioScan 920-II,	lean body mass and total water content <b>were significantly correlated to BM</b> total protein, fat, and energy (p <0.05).
Bzikowska-Jura et al. 2021	To investigate the relationship between breast milk iron and	32 women giving birth to term infants with mean age	5-10 ml of breast milk by hand expression was taken at pre and post feeding over 24 hours period at 4 time points; no	<b>Dietary Intake: using</b> self-report 3-day dietary record and FFQ; calculating	-Maternal diet (zinc and iron intake) and body fat mass <b>positively</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Poland	zinc concentrations and maternal diet and maternal BMI	of 33.8 years at postpartum age of 4 to 6 weeks	report type of storage before analysis; BM iron and zinc contents analyzed by inductively coupled plasma mass spectrometer	nutrient diet by Dieta 6.0 nutritional software <b>BMI measurement:</b> Pre-pregnancy BMI; weight gain during pregnancy; BMI during lactation; body composition assessed using the Maltron BioScan 920-II	<b>influenced</b> zinc and iron concentrations in BM ( $p < 0.05$ ).
Daniels et al.	To assess maternal	113 women giving birth to	Two aliquots of 1 ml milk samples by hand expression or	<b>Dietary Intake:</b>	<b>There was significant</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
2019  Indonesia	micronutrient intakes and their relationship with milk concentrations	term infants with mean age 25.8 years at postpartum age of 2 to 5 months	breast pump were collected in the morning on day 14; no report on timing to infant's last feeding; samples were stored at -80°C until analysis; BM micronutrient analysis used Agilent technologies	Self-report 3-day dietary record	<b>positively</b> between maternal micronutrient intakes (vitamin A, niacin, and riboflavin) and milk retinol, nicotinamide, and free riboflavin concentrations (P< 0.05).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Gibson et al.  2020  Indonesia	To investigate maternal micronutrient intake and micronutrient concentrations in BM	212 women giving birth to term infants with mean age of 28 years at postpartum age of 2 months and 5 months	Total volume pumped & collected not reported and milk was collected by hand expression pump in the morning; no report on timing of last infant feeding related to milk collection; samples were stored at -80°C; micronutrients in BM analyzed by ICP-MS	<b>Dietary Intake:</b> 3-day dietary record; calculating maternal micronutrient intake used locally produced Indonesian food composition table	<b>- Statistically significant associations</b> existed at 2 months between concentrations of calcium, iron, zinc, and niacin in BM and maternal intakes (p< 0.05).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Hascoet et al.  2019  France	To determine the impact of maternal diet on breast milk macronutrients.	81 women giving birth to preterm infants with median age of 29 years at postpartum age of 1 to 5 weeks	500 ml of milk samples was collected multiple pumping every three days during the first five weeks of lactation and no concern the time of collection; no report on type of breast milk expression and time last infant feeding; milk stored at -20 after collected and then -80°C until analysis; BM Macronutrients analyzed by Miris HMA™	<b>Dietary Intake:</b> FFQ was collected by dietician interview; calculating macronutrient intake by Micro 6 diet analyzer software.	There was a <b>positive relationship between milk protein, fat, and calorie levels and woman's carbohydrates intake</b> (p <0.05).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Huang & Hu  2020  China	To evaluate the association between breast milk macronutrient with dietary pattern among lactating women.	220 women giving birth to term infants with mean age of 27 years	20 ml of milk by electric breast pump was taken between 8:00 a.m. to 11:00 a.m.; no report on timing of last infant feeding, total volume pumped, and type of storage and temperature before analysis; BM  Macronutrients analyzed by Miris HMA™	<b>Dietary Intake:</b> 3-day dietary record; food dietary pattern was identified by exploratory factor analysis	<b>Dietary pattern with high intake of red meat, cereals, and eggs (pattern 2) was associated with higher protein, total dry matter, and energy contents in breast milk (p&lt; 0.05)</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Hu et al.  2021  China	To assess dietary patterns and their associations with BM macronutrient composition.	122 women giving birth to term infants with mean age 29.3 years at postpartum age of 52 days	10 ml of mature milk from pre-feeding by breast pump or by hand was taken between 6:00 to 10:00 a.m. at 5 visits (day 0-7, 8-13, 14-21, 22-35, and 36-51); sample put on ice immediately after expression and then stored at -80°C until analysis; BM Macronutrients analyzed by Miris HMA™	<b>Dietary Intake:</b> 24-hour and 3 days food dietary records; calculating macronutrient intake by the China Food Composition Book; maternal dietary pattern was identified by component analysis	<b>High-in-animal-foods pattern was positively associated with carbohydrates; high-in-plant-foods pattern was negatively associated with fat; and high-in-eggs pattern was weakly positively</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
					associated with protein concentration in mature milk (p <0.05)
Makela et al. 2013 Finland	To compare BM fatty acids between overweight and normal weight women.	100 women giving birth to term infants with mean age of 30 years at postpartum age of	10 ml of milk sample by hand expression was collected in the morning at 3 months PP; no report on timing to infant's last feeding and total pumping volume; sample stored at -70°C until analysis; BM fatty acids	<b>BMI measurement:</b> women were classified two groups: Overweight (BMI ≥ 25 kg/m <sup>2</sup> ) and normal weight (BMI < 25 kg/m <sup>2</sup> )	-Overweight women <b>had higher amount of saturated and lower amount of unsaturated fatty acid</b> when

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
		1 to 3 months	analyzed by gas chromatography		compared to normal weight women (P <0.05).
Nakamori et al. 2009 Vietnam	To explore the association between maternal dietary and BM concentration of iron, zinc and copper	60 women giving birth to term infants with mean age of 25 years at postpartum age of 6 to 12 months	20 ml of milk sample by hand expression was collected in the morning; no report on timing to infant's last feeding and/or total pumping volume; samples stored at -20°C; BM micronutrient analyzed by -Kjeldahl and Rose-Gottlieb method	<b>Dietary intake:</b> 3 days food dietary records; calculating maternal nutrient diet by dietary reference intakes (DRIs) in Vietnam	BM concentration of iron, zinc, and copper <b>was not correlated to the</b> maternal dietary intake of iron, zinc, and copper.

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Peng et al.  2021  China	To investigate breast milk composition and its relationship with maternal body mass index (BMI)	101 women giving birth to term infants with mean age of 29.4 years at postpartum age of 1 to 3 months	10 ml of milk sample was taken from fully pumped one breast; no report on the type of breast milk expression and time of milk collection; sample was frozen at -20°C within one week and then stored at -80°C; BM Macronutrients analyzed by Miris HMA™; fatty acid (poly unsaturated FA; PUFA) was determined by gas chromatography	<b>BMI measurement:</b> women were classified into two groups: Low BMI ( $18 \text{ kg/m}^2 \leq \text{BMI} \leq 20 \text{ kg/m}^2$ ) and High BMI ( $\geq 25 \text{ kg/m}^2$ )	-women who had <b>high BMI presented increased</b> total energy, fat, protein and PUFA in breast milk as compared with low BMI women ( $P < 0.05$ ).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Rakicioglu et al. 2006 Turkey	To determine the effect of Ramadan fasting on maternal nutrition and breast milk composition.	21 women giving birth to term infants with mean age of 27.3 years at postpartum age of 2 to 5 months	milk sample by hand expression was collected after infant feeding immediately and collected until empty breast between 09.00 and 11.00 am.; sample was stored at -70°C; BM macronutrients analyzed by micro-Kjeldahl method (for protein), YSI model 2700 (for lactose), and Roese–Gottlieb method (for fat); BM	<b>-Dietary Intake:</b> 3-day dietary record at 2 times; second week of Ramadan and 2 weeks after the end of Ramadan; calculating maternal nutrient intakes by computer program.	Zinc, magnesium and potassium levels in breast milk <b>decreased significantly</b> during Ramadan fasting.

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
			Micronutrients analyzed by Shimadzu AA-600 model		
Sims et al. 2020 USA	To examine the relationship between maternal BMI and BM macronutrients	174 women giving birth to term infants with mean age 30.4 years at postpartum age of 2weeks to 9 months	Milk sample by hand or electric pump was collected after the second infant feeding of the day in the morning at multiple time points (postnatal age 0.5, 1,2,3,4,5,6 and 9 month); no report on total volume pumped & collected; sample was stored at-70°C; BM Macronutrients analyzed by Miris HMA™	<b>BMI measurement:</b> women were categorized into two groups: normal weight (NW; BMI: 18.5-24.9) and overweight/obese (OW; BMI: 25-35)	BM in OW women were <b>higher in fat and protein and lower in carbohydrate</b> content at 4, 5, and 6 months when compared to NW women.

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Titi et al.  2014  China	To examine whether dietary or other maternal factors can affect the levels of macronutrients in breast milk	436 women giving birth to term infants with mean age 27 years at postpartum age of 1 to 8 months	40 ml of milk sample from one breast using hand expression and breast pumps was collected between 9:00 and 11:00 a.m.; samples were stored at -80°C until analysis; BM Macronutrients analyzed by Miris HMA™	<b>Dietary intake:</b> 24-hr dietary recall; calculating nutrient intake by using the 2002 China Food Composition Database. <b>BMI measurement:</b> Weight and height before and after pregnancy	-Maternal BMI was <b>positively associated with milk fat and protein content</b> and <b>negatively with lactose</b> greater than dietary intake ( $p < 0.05$ ).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
Zhang et al. (2021) Tibet	To explore the association between the Tibetan maternal diet pattern and human milk composition	50 women giving birth to term infants with mean age of 27.2 years at postpartum age of 1 month	60 ml of milk sample from one breast using electric breast pump was collected between 09:00 and 11:00 am; no report on timing to infant's last feeding; samples were stored for up to 7 days in -20°C before storage at -80°C until analysis; BM Macronutrients analyzed by Miris HMA™; BM fatty acids analyzed by gas	<b>Dietary intake:</b> 3 days of diet recorded; FFQ; maternal diet patterns were identified by factor analysis of maternal nutrient intake	- There was significant <b>correlation of protein contents</b> between <b>maternal dietary intake and BM</b> (p< 0.05) -Micronutrients in BM, including <b>unsaturated fatty acids and free essential amino</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
			chromatography-mass spectrometry (GC-MS); BM free amino acids analyzed by ultraperformance liquid chromatography (UPLC)		<b>acids</b> , were also <b>impacted by maternal diet.</b>
<b>Experimental studies investigating maternal supplement and macro/micronutrient composition in breast milk</b>					
Argaw et al. (2021) Ethiopia	To evaluate the efficacy of fish-oil (FO) supplementation of lactating women on BM	129 women giving birth to term infants with mean age of 25.9 years at postpartum	9 ml of milk sample by hand expression or breast pump was collected after the women breastfed for a “few minutes to establish breastfeeding”; no report of milk collection time;	<b>Maternal supplement:</b> -women of intervention group received FO capsule (500mg/d of polyunsaturated fatty	- BM concentration of DHA and EPA was 39% and 36.2% <b>higher (respectively) in</b>

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	long-chain polyunsaturated fatty acids (LCPs), including DHA and EPA concentrations.	age of 6 to 12 months	samples stored at -80°C until analysis; BM LCPs analyzed by Bligh–Dyer method	acids); control group received corn-oil (CO) capsules during 12 months.	<b>the FO group</b> compared to the CO group (p< 0.001).
Fidler et al. 2000 Germany	To evaluate the effects of DHA supplementation on the fatty acid	10 women giving birth to term infants with mean age 29.2 years at	milk by electric breast pump was collected in the morning at 4 weeks PP, and every 6-12 hours over 48 hours at 6 weeks PP (after ingesting the study	<b>Maternal supplement:</b> women in intervention group received 2 capsules/day,	DHA concentration in BM was <b>significant higher</b> in the intervention

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	composition of BM	postpartum age of 4 to 6 weeks	supplement); no report on total volume collected; sample stored at -80°C until analysis; BM fatty acid analyzed by gas-liquid chromatography	containing oil rich in DHA (DHASCOTM, 200 mg of DHA/day), while control group received placebo oil during 14 days.	group compared to controls. (p< 0.003).
Mazurier et al. 2017 France	To evaluate the effect of maternal supplement of omega-3 PUFAs during	80 women giving birth to term infants with mean age of 31.5 years at postpartum	10 ml of milk sample after first infant feeding of the day was collected in the morning at day 0, 15, and 30; no report on the type of breast milk expression; samples were stored	<b>Maternal supplement:</b> -women were randomized to one of four groups:	BM fatty acid was <b>significantly higher after diet supplementation</b> with omega-3 PUFAs (group

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
	15 days on fatty acid (FA) compositions in BM.	age of 1 to 4 months	in the freezer at -18 °C before storage at -20°C until analysis; - BM fatty acid composition analyzed by gas chromatography	olive oil group (O group, control), an omega-3–enriched margarine group (M group), a rape- seed oil group (R group), and an omega-3–enriched margarine plus rapeseed oil group (MR group).	MR, R, and M) when compared to non-omega-3 PUFAs (group O) (p<0.003).

Author, year, country	Study design and aims	Sample size and Participant characteristic	Milk sample and analysis	Maternal nutritional assessment/tool	Major findings
				-Each group received supplements twice per week.	

## **CHAPTER 3**

### **RESEARCH METHODOLOGY**

#### **Introduction**

This study used a non-experimental correlational study design to explore the impact of maternal nutrition factors and other relevant factors during the first month of lactation on macronutrient composition in breast milk among Thai mothers of premature infants. Forty-seven mothers who give premature birth were selected according to convenience purposive sampling. Current maternal dietary intake of macronutrients was collected from 2 non-consecutive days from 24-hour dietary food recalls (one day during the week and one weekend day). The habitual diet intake of mothers was collected from food frequency questionnaires (FFQs). Maternal body mass index (BMI) was evaluated with body weight and height measurement and calculated with the standard equation: weight/height (kg/m<sup>2</sup>). Breast milk sampling was done at two-time points; time point 1 (during 1-2 weeks of lactation) and time point 2 (during 3-4 weeks of lactation). A MIRIS human milk analyzer was used to assess breast milk macronutrients. This chapter provided detail on (1) the study design, (2) the population and sample, (3) the study setting, (4) the study measurements, (5) the data collection procedure, (6) data analysis planning, (7) feasibility of data collection, and (8) ethical considerations.

#### **3.1 Study Design**

The non-experimental correlational design was used in this study with the intention to examine the association of maternal nutrition factors (current diet intake of macronutrients, habitual diet based on frequency of food consumption, current BMI),

maternal demographic factors (maternal age, education level, multiple pregnancies mode of delivery), and infant factors (gestation age and sex) with the macronutrients in mature breast milk among mothers of preterm infants in Thailand. The purpose of a non-experimental correlational study is to understand relationships among variables without manipulating the independent variables (Polit & Beck, 2017). This design was used because manipulating independent variables could not be conducted ethically. In this study, controlling maternal diet, weight, and height to assess their relationship with breast milk macronutrients could cause physical or mental harm to mothers, leading to unethical issues. Non-experimental correlational design was appropriate for this study.

### **3.2 Population and Sample**

The population of this study is mothers who give birth to premature infants in Thailand. The sample is defined as Thai mothers of preterm infants who are recruited to this study according to the inclusion criteria. Purposive convenience sampling was used to select eligible participants in this study due to feasibility and economical considerations. Additionally, purposive sampling focuses on particular characteristics of a population that are of interest, which will help researchers better answer research questions. This sampling method also provides justification to make generalizations from the sample (Etikan et al., 2016).

#### **3.2.1 Inclusion and Exclusion criteria**

To be eligible for participation in this study, mothers of preterm infants (1) gave birth between 30-36 weeks of gestation and their infants admitted to sick newborn unit, (2) have age  $\geq$  18 years old, (3) are able to speak Thai, (4) have a LINE application (a

free messaging and voice/video calling application) to communicate with the researcher, and (5) can provide breast milk sample from an electric pumping session. Mothers were excluded if (1) they were unable to provide breast milk for any reason during the study, (2) they have any chronic diseases to affect their nutrition (diabetes, hypertension, cancer) and (3) they smoke.

### 3.2.2 Sample size

The sample size was determined based on power analysis with G-power software, a free power analysis software used to calculate the adequate size of the study sample to test research hypotheses (Kang, 2021). According to G-power analysis, this study was conducted with a level of significance ( $\alpha$ ) of .05, power of .80. The effect size was calculated based on previous study as follows:

1. Based on a study conducted by Yang and colleague (2014) compare the breast milk energy and macronutrient concentrations of healthy urban Chinese mothers at different lactation stages. The result report that fat levels among those in the 5-11 days group significantly differed from those in the 12-30 days group. Hence, the Cohen's effect size (d) is determined by calculating the mean difference value of fat content between the 5-11 days and 12-30 days group and then dividing the result by the pooled standard deviation.

$$d = M_2 - M_1 / \sigma$$

$$\sigma = \sqrt{(SD_1^2 + SD_2^2) / 2}$$

d = the effect size,  $m_2 - m_1$  = the mean difference between two groups,  $\sigma$  = the pooled standard deviation

Day 5-11 group;  $M_1 = 2.9$ ,  $SD_1 = 1.1$

Day 12-30 group;  $M_2 = 3.6$ ,  $SD_2 = 1.4$

$$d = 3.6 - 2.9 / \sqrt{(1.1)^2 + (1.4)^2 / 2}$$

$$= 0.56$$

Calculation from the G-power analysis for t-test: Mean difference between two dependent means (effect size = 0.56, power of test value at .80, and  $\alpha$  of .05.). The sample is 22.

2. For Cohen's effect size ( $f^2$ ), since this study examine the relationship between multiple independent variables, including maternal nutrition factors (diet and BMI), maternal demographic factors (maternal age, education level, multiple pregnancies, mode of delivery), and infant factors (gestational age and infant gender) and their impact on breast milk macronutrient, the effect size ( $f^2$ ) was calculated from relevant previous studies as follows:

2.1 Based on study by Borràs-Novell et al. (2023) examine the effect of maternal and perinatal factors on macronutrient content of very preterm human milk during the first weeks after birth. Advanced maternal age, gestational age, multiple pregnancies had significant effects of 32.6% as  $R^2 = 0.326$  on breast milk protein. For the Cohen's  $f^2$  is determined by  $f^2 = R^2 / (1 - R^2)$ .  $f^2 = 0.48$

2.2 Based on Burianova et al. (2019) determine the relationship of maternal-associated factors on the content of macronutrients in human milk for the first six weeks after preterm delivery. The mode of delivery had significant effects of 20.9% on protein levels in breast milk as  $R^2 = 0.209$ . Calculation for the Cohen's  $f^2$  is determined by  $f^2 = R^2 / (1 - R^2)$ .  $f^2 = 0.26$ .

2.3 based on a previous study conducted by Hascoet et al. (2021)

determine the factors affect mothers' milk macronutrients after premature delivery. The maternal diet intake of carbohydrate and gestational age had significant effects of 29.9% as  $R^2 = 0.299$  on breast milk protein. Thus, the Cohen's  $f^2$  is determined by  $f^2 = R^2 / (1 - R^2)$ .  $f^2 = 0.43$ .

Thus, the average Cohen's effect size ( $f^2$ ) is equal to  $0.48+0.26+0.43/3= 0.39$

Calculation from the G-power analysis for F-test: Linear multiple regression (effect size = 0.39, power of test value at .80, and  $\alpha$  of .05., number of predictors 8). The sample is 47 as shown in figure below

To generalize the population from the study finding and reduce a threat to external validity, the researcher has to select a larger sample size (Groove, Burns & Gray, 2013). Therefore, this study was recruited a total of 47 subjects. To prevent attrition from the sample size during the study, approximately 10% is added. Thus, the required sample size was 52 samples.

### **3.2.3 Recruitment sample**

The following recruitment sample procedures were conducted: 1) After obtaining IRB permission, the researcher contacted head nurses and staff nurses of the sick newborn unit to provide study details, including the study purpose, procedures, and expected outcomes. These nurses helped facilitate the data collection process during the study. 2) The researcher collaborated with the nurses working in the sick newborn unit to obtain a list of potentially eligible mothers for participation in the study by reviewing the admission notebook and the medical chart, which is the record of mothers and premature infants who are admitted to the sick newborn unit. 3) Mothers of preterm infants who are

eligible were contacted by the principal investigator face to face to invite participation in the study within the first week after delivery. Prior to an invitation to participate in this study, the researcher conducted an assessment to evaluate the readiness of the mothers to receive information. This assessment involved observing facial expressions, eye contact, and gestures while also inquiring about any feelings of fatigue resulting from the delivery. After assessing the readiness of the mothers and confirming their willingness to participation, the researcher introduced herself and clarify the study objective and the data collection procedure to potential mothers. Also, the researcher let mothers read the informed consent, ask questions, and feel free to decide on participation in the study. 4) After the mothers agree to participate in the study and sign the consent form, the researchers collected contact information, including LINE ID, and make arrangements for collecting milk samples, dietary intake, and BMI. The recruitment procedures were stopped when the study meets the required sample size.

### **3.3 Research setting**

The study was conducted at a 24-bed sick newborn unit from Ramathibodi Hospital, Thailand. Ramathibodi Hospital is one of the excellent tertiary cares and a university teaching hospital that provides multidimensional care for over 500 preterm and sick infants per year in Thailand. Because the hospital's capability that provide care with the tertiary level, it allows many mothers of premature infants across Thailand can come to this hospital. Consequently, this research setting can offer the possibility of sample recruitment and represent target population for this study. In the sick newborn unit, there is a breastfeeding room that provide space for mother to express breast milk for their

infants. This room allows mothers to use their own breast pump or electric breast pump provided by the unit. In case of mothers who cannot afford an electric pump, the unit offers ten electric breast milk pumps in the breastfeeding room to facilitate their breast milk expression. Also, these pumps can be borrowed by the mothers and taken home until their infant is discharged from the hospital. The characteristic of this setting provide feasibility of collecting breast milk for this study.

### **3.4 Measurements**

This study has four parts of measurements: maternal dietary intake, anthropometric measurements for maternal body mass index (BMI), demographic data of mother and preterm infant, and breast milk component analysis. The details of each measurement was described as follows:

**1. The maternal dietary intake:** It refers to the consumption of a nutrient or food in mothers during the first month of the lactation period. Maternal dietary intake was evaluated by two different measurements. The first measurement is current maternal diet intake of macronutrients, which is assessed by a 24-hour food recall record. The second measurement is mother's dietary habit over the past month, which is assessed by Food Frequency Questionnaires (FFQs). Then, the macronutrient intake of mothers was analyzed by INMUCAL nutritional analysis software.

**1.1) 24-hour food recall record (see Appendix A):** The researcher created this recall record based on the U.S. Department of Agriculture's Automated Multiple-Pass Method food recall (AMPM). The AMPM is the standardized dietary assessment approach designed to collect detailed information on an

individual's food and beverage consumption in the previous 24 hours (Raper et al., 2004). The AMPM can be used as interviewer-administered either in person or remotely. This method has been widely validated in many studies for complete and accurate food recall and reduces the respondent burden with numerous memory cues integrated into the questions (Moshfegh et al., 2008; Steinfeldt et al., 2013). For this reason, the researcher applied this standardized food recall approach to create a 24-hour food recall record. Although this food recall approach has been translated in many languages including Spanish, Portuguese, French, Chinese, and Korean, a Thai version is currently unavailable. To collect maternal diet intake from this study, this recall record was created from English and translated into Thai version. To increase validity of the food recall record, a process of back-translation with expert was conducted. Initially, the researcher forward-translated the food recall record from English to Thai language. Subsequently, two bilingual experts in the nursing field independently translated the Thai version back into English. The researcher then compared for the similar word and meaning between the back-translated and the original version. If any discrepancies in meaning were identified, the process was repeated again until both versions were aligned in meaning. The purpose of this food recall record is to collect data about all foods and beverages that mothers consumed in the previous 24 hours. The researchers completed this record using the LINE application, a popular communication application in Thailand. LINE provides free messaging, voice, and video calls. So, the mother can interview the researcher

using video calls and send food pictures to researchers to check accurate food and drink consumed. This food recall record was conducted for 2 non-consecutive days; 1 day during the week and 1 weekend day since many individuals eat differently during the week and on the weekend. The record consists of two sections. The first section is instructions on form completion following the five steps employed in the AMPM approach. **Step 1:** “Quick list” (the record form started by asking all the list of foods and drinks consumed during the previous 24 hours); **Step 2:** “Forgotten foods” (probe questions about foods and drinks was conducted to help the mother remember foods in case they forgot); **Step 3:** “Time & Occasion” (time, place, meal, and occasion for each food were collected in the record form); **Step 4** “detail cycle” (detailed food description and amount of each food were recorded in the form) and **Step 5:** “Final Probe” (the researcher reviewed and checked accurate information before completing the form). The second section is a table of food record. The researcher filled out foods and drink data following the instruction part.

- 1.2) Food Frequency Questionnaires (FFQs) (see Appendix B):** This food questionnaire is developed by the researchers adapted from Diet History Questionnaire (DHQ) III-Food Frequency Questionnaire, which is a freely available food frequency questionnaire (FFQ) for use with adults 19 or more years of age by the National Cancer Institute (Subar et al., 2001). The DHQ III questionnaires are currently accessible only in English and Spanish. Additionally, there are few available tools for evaluating diets in Thailand,

with only one FFQs identified in the literature for assessing individuals at risk of metabolic syndrome (Nirdnoy et al., 2023), which may not be appropriate for this research. Due to limited nutrition assessment, this FFQs based on DHQ III questionnaires was created to gather more data on maternal diet intake. To increase validity of assessment maternal diet intake in this study, FFQs are designed to assess habitual diet of mothers by asking about the frequency with which food items or specific food groups are consumed over the past month during lactation. It provides a more comprehensive assessment of an individual's dietary intake and reduces the impact of day-to-day variations in food consumption rather than food recall record (Food and Agriculture Organization of the United Nations, 2018). The list of foods in the questionnaires were applied from the food composition table of Thai food, which includes 13 food groups as follows: i) cereal and their product; ii) Starchy roots, tubers, and their product; iii) Pulses, seeds, nuts, and their products; iv) Vegetables and their products; v) Fruits and their products; vi) Meat, poultry, and their products; vii) Finfish, shellfish, other aquatic animals and their products; viii) Eggs; ix) Milk and its products; x) Spices and condiments; xi) One plate dishes, main dishes, local foods, and vegetarian foods; xii) Desserts and bakeries; and xiii) Insects/ Miscellaneous (Judprasong et al., 2018). The response options were arranged in five categories that are modified from the DHQ questionnaires from "never," "less than once a week," "once or twice a week," "more than twice a week but not every day," to "every day." The questionnaires include two parts; the first part has instructions, and

the second part is a list of food items. The FFQs were collected from the mother using interview by LINE application at the single time, during the 3-4 weeks of lactation period.

- 1.3) INMUCAL nutritional analysis software:** The energy and nutritional value of maternal diet intake were calculated using INMUCAL-Nutrients V.4.0, a nutritional analysis software developed by the Institute of Nutrition, Mahidol University, Thailand. This nutritional software includes more than 30 kinds of food and 15 types of nutrients that are typically consumed in Thailand to calculate personal nutrient intakes (Jeong et al., 2021). The INMUCAL-Nutrient software program converts raw food intake to nutrients content, including macronutrients (such as protein, fat, and carbohydrates) and micronutrients (such as vitamins and minerals), based on the Thai Dietary Reference Intake (DRI) (Banjong et al., 2016). The food and drink data from the 24-hour food recall record were imported to this software for analysis of the energy and nutrients of maternal diet intake.
- 2. Anthropometric measurements for maternal body mass index (BMI):** This measurement is defined as the current weight and height of the mother during the first month of lactation. The maternal weight and height were measured using a standing digital scale and portable stadiometer. To ensure accuracy of measurement, the scale was calibrated daily, and the mothers were requested to wear minimum clothing or other items that could alter their weight as recommended by World Health Organization (de Onis & Habicht, 1996). All measurements were done in duplicate. Then, maternal weight and height were calculated to the body mass index (BMI)

which was calculated by squaring the height in meters and then dividing the weight by height in meters squared ( $\text{kg}/\text{m}^2$ ). All data was recorded in the maternal body mass index (BMI) record form (See Appendix C).

3. **Demographic data of mother and preterm infant:** This measurement refers to a group of demographic data factors of mothers and infants, including maternal age, educational level, multiple pregnancies, mode of delivery, gestational age, and infant sex as described by definition in chapter 1. All data was collected during the first two-weeks of lactation from the medical chart and recorded in the demographic data form developed by the researcher (See Appendix D).
4. **Breast milk component analysis:** The components of breast milk in this study refers to macronutrient concentration, including carbohydrates, protein, fat, and energy during the first month of lactation. MIRIS human milk analyzer (HMA) was used to measure breast milk macronutrient composition in this study. The detail of this tool is described below:

4.1) **MIRIS human milk analyzer (HMA)** is a tool to analyze the nutritional content of breast milk. It uses mid-infrared (MIR) transmission spectroscopy, the certified method proven to be reliable for milk analysis (Smilowitz et al., 2014). The concentration of all breast milk macronutrients was reported in grams (g) per 100 ml of milk, and energy values was reported in kcal per 100 ml. The carbohydrate levels reported in MIRIS HMA include the content of 70% lactose and 30% oligosaccharides of breast milk. The crude protein includes all protein and nonprotein nitrogen sources, and true protein cover only the protein sources of nitrogen. The fat concentration refers to total lipid-

soluble in breast milk, such as triglycerides, diglycerides, free fatty acids, phospholipids, and cholesterol. Before analysis, each milk sample was warmed to 37-40°C and homogenized. A 3-ml milk sample was analyzed during each measurement using MIRIS HMA. A zero-setting check and calibration with the standardized solutions procedure were implemented routinely at startup and after cleaning the instrument (Miris, 2020).

### **3.5 Data collection procedure**

The data collection procedure was implemented at two time points according to lactation stage of breast milk during the first month of lactation: 1<sup>st</sup> Time point at colostrum & transitional milk (during the first 1-2 weeks of lactation), and 2<sup>nd</sup> Time point at mature milk (during 3-4 weeks of lactation).

#### **1. 1<sup>st</sup> Time Point (between 1-2 weeks of lactation)**

- 1.1 The socio-demographic and maternal characteristics, such as age, educational level, multiple pregnancies, mode of delivery, gestational age, and infant sex were collected from the medical chart and record in the demographic data form.
- 1.2 All Mothers were provided with a comprehensive kit consisting of a 10 ml container, an insulated carrying bag, and a gel freezer pack. The kit came with instructions on how to collect, store, and transfer their breast milk sample for analysis. Each mother was instructed to collect 3-9 ml of breast milk from one fully pumped breast using an electric breast pump and storing it in a 10 ml container. MIRIS HMA recommends using 3 ml of milk per analysis, and

therefore, a total of 9 ml is required for triplicate milk analysis. However, if a mother is unable to produce the required amount of milk, the requested volume can be adjusted to either duplicate analyses using 6 ml in total, or at least a single analysis using 3 ml. Mothers were instructed to “fully” pump their breasts and then the milk sample was taken from the full sample from one breast in the morning after breakfast and before lunch (7.00 AM – 11 AM). This collection of breast milk within the specified timeframes is to minimize possible circadian influence on breast milk macronutrient composition. The mothers were instructed to refrigerate milk samples (~4°C) at home and bring them to the sick newborn unit in an insulated carrying bag within 24 hours after collection. Then, all samples was transferred to Ramathibodi Human milk bank and stored in refrigerator before analysis.

1.3 Milk samples was analyzed by using MIRIS HMA within 7 days after receipt of the samples. All sample was measured fresh or refrigerated to avoid the effect of freezing and rewarming sample on breast milk compositions.

## **2. 2<sup>nd</sup> Time Point (between 3-4 weeks of lactation)**

2.1 Researchers completed the 24-hour food recall record from the mothers using video calls from the LINE application. The data was collected for 2 non-consecutive days; 1 day during the week and 1 weekend. All food and drink data were recorded in the food recall record following instruction. To ensure reliable of food and nutrient intake, mothers was requested to send their food pictures. Also, local household measurements such as rice serving spoons, cups, dishes, tablespoons, or teaspoons was measured during the interview to

verify the amount of food/beverage based on the food base dietary guidelines developed by Department of Health, Ministry of Public Health, Thailand. It commonly uses household utensils found in the kitchen to measure the portion size of foods and beverages. Then, the researcher entered the data into INMUCAL nutrient software for analysis.

2.2 After collecting maternal diet intake, the mothers were requested to collect breast milk sampling again. The detail of breast milk collection and breast milk analysis was implemented as the same step of 1.2, 1.3 data collection procedure.

2.3 At the same day of milk collection, mothers of preterm infants were measured for their body weight and height. All measurements were conducted twice. Maternal weight was measured using a digital scale and height was measured using a stadiometer. Maternal body mass index (BMI) was calculated and recorded in the form. Also, the research collected pre-pregnancy weight from medical chart and recorded in the form.

2.4 Also, the research completed Food Frequency Questionnaires (FFQs). The mother recalled the consumption frequency of food in the past month.

The data collection procedure was summarized in the figure 2 below.

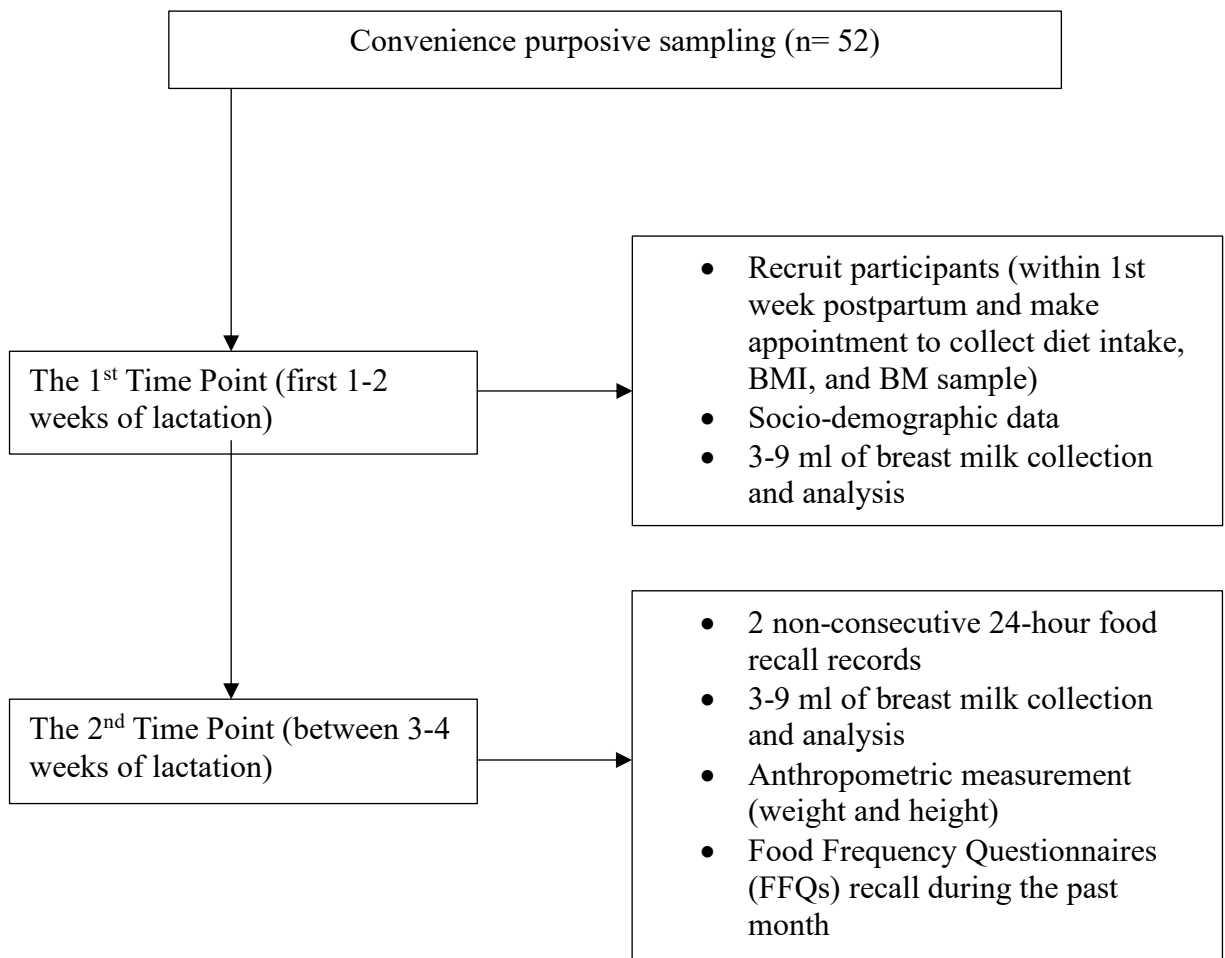


Figure 2: Summary of Data Collection Procedure

### 3.6 Data analysis planning

The data analysis was performed using the Statistical Package for Social Science version 29 (SPSS) with the statistical significance set at  $p < 0.05$ . The characteristics of the study sample, maternal macronutrient intake, dietary habits, and nutritional status were analyzed using descriptive statistics such as mean, standard deviation (SD), frequency, and percentage. To compare breast milk macronutrients at different stages of

lactation during the first month of lactation (during 1-2 weeks vs. during 3-4 weeks of lactation), the Wilcoxon signed ranks test was employed due to the non-normal distribution of the data. In order to explore the impact of maternal nutrition factors and other factors on the level of macronutrients in mature milk, multiple linear regression models were conducted. Since macronutrients in mature milk include fats, carbohydrates, energy, and proteins, this study created separate models for each macronutrient in mature milk (BM fats, BM carbohydrates, BM energy and, BM proteins) as the dependent variables and the maternal nutrition factors (current diet intake of macronutrient, frequency of food consumption, current BMI), maternal demographic factors (maternal age, education level, multiple pregnancies, mode of delivery), and infant factors (length of gestation and infant sex) as the independent variables. Additionally, the multiple regression analysis was conducted in a backward manner with independent variables that have  $p$ -values  $<0.05$  was selected for inclusion into the final regression model.

### **3.7 Feasibility of data collection**

The timeline of this study was presented in Figure 3 below. It was anticipated that the study may face challenges in recruiting participants according to several inclusion criteria for selecting the sample. This process took a longer time to find eligible participants. Based on statistics reported in the Ramathibodi Hospital during 2018-2022, infants born prematurely were 521, 495, 522, 623, and 426 cases per year, respectively. It can estimate that the mothers who give premature birth were admitted to Ramathibodi Hospital between 35-51 cases per month. This number provides enough potential participants for the study, which aims to recruit a total of 47 samples. This study took 6

months to complete data collection. In addition, to meet the criteria for milk sampling, which involves collecting breast milk using an electric breast pump, the study site can provide ten electric breast pumps to mothers who do not possess them. The availability of these electric pumps can increase the practicality of collecting breast milk samples for this study. Regarding challenging collaboration with nurse staff in the unit, lacking of understanding of the study detail among staff can occurred. This issue was made a difficulty in the data collection process. To overcome this challenge, the primary researcher communicated with the head nurse and staff to inform them about the study aims, expected outcomes, and data collection procedure. Also, the good relationship between the primary researcher and nurse staff in the unit can improve this challenge. Since the researcher was previously a member of the nursing team in this unit, all the head nurses and staffs are familiar with her. Finally, the challenge of time commitment from the participant was expected. The participant would face this challenge, as much data was collected at the two-time points (during the first 1-2 weeks of lactation and 3-4 weeks of lactation). In anticipation of this burden, the following strategies was employed. First, the researcher face by face contacted and provided contact with the participant for communication. The data collection date was selected by the participant that is most convenient for them. However, it should occur due to the study timeline (between 1-2 weeks and 3-4 weeks of lactation). Second, the researcher provided gift cards to acknowledge the participant's time. The gift card was provided after milk collection at the first and second-time points, respectively. Also, some participants were difficult to send breast milk samples due to insufficient transportation for sending the samples to the

study site within the specified timeframe. In this case, the research used messenger delivery to collect milk sample from mother.

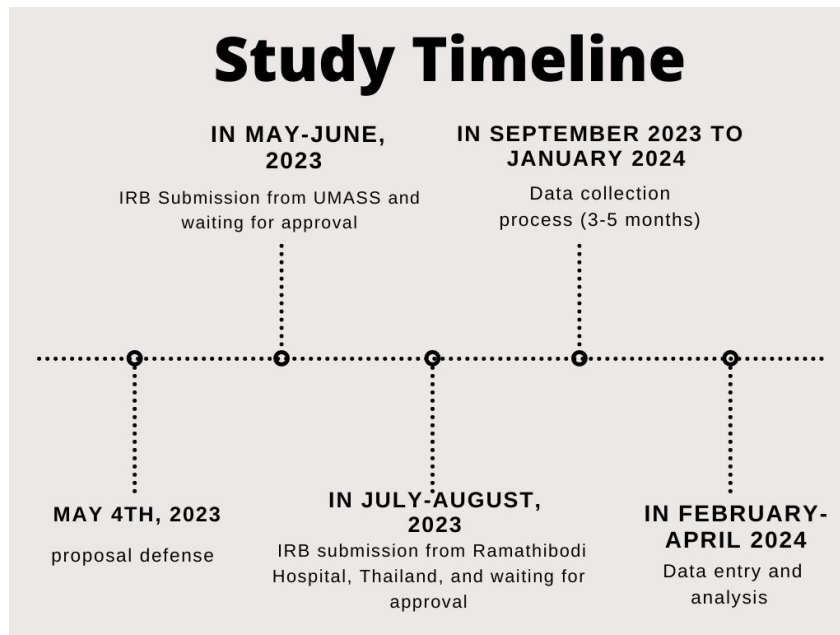


Figure 3: The study timeline

### 3.8 Ethical considerations

The research was submitted to the Institutional Review Board (IRB) of the University of Massachusetts Amherst and the Human Research Ethics Unit, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand, to be reviewed for compliance with ethical principles. After receiving approval from IRB, the data collection procedure was begun. All participants were asked to sign a consent form before enrolling in the study. Obtaining consent was continuous, and the consent form provides detailed information about all parts of the study. Moreover, the participants were informed that they have the right to withdraw from the study at any time if they feel uncomfortable. All participant data was de-identified and labeled by study I.D. number only. The data

storage security was maintained; electronic data was password protected, and accessible only to the researcher and research staffs. After finishing study, data was stored for the length of time recommended by IRB. Study findings were presented in summary format so that individual participants were not identifiable.

### **3.9 Summary**

In summary, this chapter describes the methodological approach in this study. Non-experimental correlational design was conducted to examine research aims. Convenience purposive sampling was implemented to recruit study sample. All measurements, including maternal diet intake, maternal BMI, demographic data of mother and preterm infant, and breast milk analysis component, was delineated in this chapter. The data collection process was explained with details about every step taken, from sample recruitment to data collection procedure. Finally, the data analysis planning and the means of addressing ethical considerations in this study were presented.

## CHAPTER 4

### RESULT

#### 4.1 Introduction

This chapter presents the study findings, organized into three sections according to the research aims. For each section, the research aim is outlined, and the detailed findings are presented. The summary of demographic characteristics of the study sample is also presented in this chapter before the detailed study findings.

#### 4.2 Demographic of Study Sample

A total of 52 mothers meeting the inclusion criteria of infant gestational age between 30-36 weeks, age over 18 years, spoke Thai, had the LINE application, and could provide breast milk using an electric breast pump were initially enrolled in this study between October 2023 and March 2024. Of these, five mothers were withdrawn from the study due to incomplete data collection, resulting in a final sample size of 47 mothers of preterm infants for the final analysis.

The demographic characteristics of the study sample are summarized in Table 2. The mean value of maternal age was  $33.5 \pm 5.9$  years. The majority of participants had attained a bachelor's degree education (n=24, 51.1%). Most mothers experienced a single birth (n=37, 78.7%) and delivered via cesarean section (n=38, 80.9%). The average gestational age was  $32.7 \pm 1.7$  weeks. Among the 47 mothers participating in the study, 26 (55.3%) had a female infant, while 21 (44.7%) had a male infant.

**Table 2** shows the demographic of the study sample (n=47)

Demographics of the study sample	Mean± SD	Number (Percentage)
Age (year)	33.5 ± 5.9	(20-47)
Gestational age (week)	32.7 ± 1.7	(30-36)
Education level		
• High school		5 (10.6%)
• Bachelor's degree		24 (51.1%)
• Beyond bachelor's degree		14 (29.8%)
• Other (diploma)		4 (8.5%)
Multiple pregnancies		
• Single birth		37 (78.7%)
• Twin birth or more		10 (21.3%)
Mode of delivery		
• Vaginal		9 (19.1%)
• Cesarean Section		38 (80.9%)
Infant gender		
• Female		26 (55.3%)
• Male		21 (44.7%)

### 4.3 Finding by study aim

**4.3.1 Aim 1:** *To compare the breast milk macronutrient components at different stages of lactation (colostrum & transitional milk: 1-2 weeks of lactation) vs. (mature milk: 3-4 weeks of lactation) and characterize the changes in macronutrients from colostrum & transitional to mature milk among Thai mothers of preterm infants.*

Table 3 provides a detailed comparison of breast milk macronutrient profiles from two different stages of lactation (colostrum & transitional milk: 1-2 weeks vs. mature milk: 3-4 weeks). The median macronutrient concentrations during 1-2 weeks were 3.8 (3.4,4.5) g/dl for fats, 7.2 (7.0,7.4) g/dl for carbohydrates, 72.7 (67.3,80) kcal/dl for energy, and 1.8 (1.7,2.0) g/dl for true proteins. While the median macronutrient levels

during 3-4 weeks were 4.2 (3.7,4.8) g/dl for fats, 7.5 (7.1,7.6) g/dl for carbohydrates, 76.7 (71.3,81.0) kcal/dl for energy and 1.5 (1.4,1.7) g/dl for true proteins.

Wilcoxon Signed Rank test was performed to compare the macronutrient concentration between breast milk during 1-2 weeks and 3-4 weeks. The results showed that carbohydrate and protein levels in breast milk significantly differed between colostrum & transitional and mature milk during the first month of lactation.

Carbohydrate content in breast milk increased during the transition to mature milk in the first 3-4 weeks of lactation. The median carbohydrate level was significantly higher in mature milk than in colostrum & transitional milk ( $Z = -3.511, p < .001$ ). Conversely, protein concentration in breast milk decreased in the mature milk (the third and fourth weeks of lactation). The median true protein concentration was significantly lower in mature milk than in colostrum & transitional milk ( $Z = -5.155, p < .001$ ). However, there were no significant differences in breast milk fat and energy content between colostrum & transitional and mature milk (fat:  $Z = -1.96, p = .05$ ; energy:  $Z = -1.498, p = .134$ ).

**Table 3** Comparison of breast milk macronutrient concentration between colostrum/transitional and mature milk using Wilcoxon signed rank test (n=47)

<b>Breast milk macronutrients</b>	<b>Colostrum /transitional milk (1-2 weeks)</b>	<b>Mature milk (3-4 weeks)</b>	<b>Z</b>	<b>p-value</b>
<b>Median (Interquartile Ranges)</b>				
Fats (g/dl)	3.8 (3.4, 4.5)	4.2 (3.7, 4.8)	-1.960	.05
Carbohydrate (g/dl)	7.2 (7.0, 7.4)	7.5 (7.1, 7.6)	-3.511	<.001*
Energy (kcal/dl)	72.7 (67.3, 80)	76.7 (71.3, 81.0)	-1.498	.134

<b>Breast milk macronutrients</b>	<b>Colostrum /transitional milk (1-2 weeks)</b>	<b>Mature milk (3-4 weeks)</b>	<b>Z</b>	<b>p-value</b>
<b>Median (Interquartile Ranges)</b>				
True proteins (g/dl)	1.8 (1.7, 2.0)	1.5 (1.4, 1.7)	-5.155	<.001*

\*Significant p-value less than .05

**4.3.2 Aim 2:** *To describe maternal macronutrient intake (based on 24-hour food recall record), dietary habits (based on intake frequency of food products), and nutritional status during the first month of lactation among Thai mothers of preterm infants.*

*4.3.2.1 Daily energy and macronutrient intake of mothers based on food recall record*

Table 4 shows the daily energy and macronutrient intake of mothers. The average nutrient intake was  $2,123 \pm 365$  kcal/day (range: 1,187- 2,990 kcal/day) for total energy intake, with  $217 \pm 44$  g/day (range: 145-317 g/day) for carbohydrates,  $91 \pm 23$  g/day (range: 39-133 g/day) for fats, and  $110 \pm 31$  g/day (range: 48-196 g/day) for protein intake. The energy intake distribution was primarily from carbohydrates (41%), followed by fats (38%) and proteins (21%), respectively.

When compared to the Dietary Reference Intake for Thais 2020, most mothers had higher average intakes than recommended for sugar (73 g/day, recommended: 24 g), sodium (3,518 mg/day, recommended: 525-1,550 mg), cholesterol (559 mg/day, recommended: 300 mg), and saturated fatty acid (26 g/day, recommended: 20 g/day). Most mothers (64%, n=30) had an average energy intake lower than the recommendation

for Thai lactating women (2,123 kcal/day, recommended: 2,280 kcal/day). However, most mothers had a higher fat (75%, n=35) and protein intake (85%, n=40) than recommended.

**Table 4** Daily energy and macronutrient intake among mothers of preterm infants (n=47)

<b>Nutrients intake</b>	<b>Average value</b>	<b>Range</b>	<b>Dietary recommendation (Adult age 19-50 years) *</b>
<b>Energy (kcal/day)</b>	2,123	1,187- 2,990	2,280 (1,780 for non-lactating women and 500 is added for lactation)
<b>Carbohydrates (g/day)</b>	217	145-317	173-414 g based on physical activity or 45-65% of total energy intake
<b>Fat (g/day)</b>	91	39-133	Approximately 78 g or 20-35% of total energy intake
<b>Protein (g/day)</b>	110	48-196	72 (53 for non-lactating women and 19 is added for lactation) or 10-15% of total energy intake
<b>Sugar (g/day)</b>	73	25-177	Approximately 24 g or not more than 6 teaspoons
<b>Sodium (mg/day)</b>	3,518	1,563-6,290	525-1,550 mg
<b>Cholesterol (mg/day)</b>	559	133- 1402	300 mg
<b>Total Saturated Fatty acid (g/day)</b>	26	8-46	Approximately 20 g or Not more than 10% of total energy intake

\*Note based on Bureau of Nutrition, Department of Health, Ministry of Public Health. (2020). *Dietary Reference Intake for Thais 2020*. <https://www.thaidietetics.org/wp-content/uploads/2020/04/dri2563.pdf>

#### 4.3.2.2 Maternal Dietary Habits Based on Food Frequency Questionnaires

Table 5 shows maternal dietary habits based on 13 food items consumed during the past month of lactation. Most mothers (78.7%, n=37) consumed cereal and cereal products every day. Starchy roots, tubers, and their products were mainly consumed less than once a week. Pulses, seeds, nuts, and their products were primarily consumed once

or twice a week. The majority of mothers consumed vegetables (83%, n=39), fruits (46.8%, n=22), meat (93.6%, n=44), eggs (40.4%, n=19), and milk (57.4%, n=27) every day. Seventeen participants (36.2%) consumed fish, shellfish, and other aquatic animals and their products once or twice a week. For spices and condiments, eighteen mothers (38.3%) used spices in their meals every day. One-plate dishes and local foods were reported as being consumed mostly more than twice a week but not every day by participants (25.5%, n=12). In this study, one-plate dishes refer to cooked-to-order meals served on a single plate, typically including a mix of carbohydrates, proteins, and vegetables. These convenient meals can be purchased from many restaurants in Thailand. Also, local foods refer to the traditional and characteristic dishes that are specific to different regions within Thailand. For example, "Som-Tum" (a spicy green papaya salad) is a local food from Northeastern Thailand, and "Khao Soi" (A curry noodle soup) is a local food from the Northern part of Thailand. For desserts and bakeries, fifteen mothers (31.9%) reported consuming them once or twice a week. Most of mothers never consumed insects during the past month of lactation (95.7%, n=45).

**Table 5** Average intake frequency (%) of 13 food list items among mothers of preterm infants during the first month of lactation (n=47)

Food items	Average frequency intake past month (%)				
	Never	Less than once a week	Once or twice a week	More than twice a week but not every day	Everyday
1. Cereals and their products, e.g., rice, bread	0%	0%	6.4%	14.9%	78.7%
2. Starchy roots, tubers, and their product	27.7%	40.4%	25.5%	6.4%	0%
3. Pulses, seeds, nuts and their products	12.8%	14.9%	48.9%	17%	6.4%
4. Vegetables and their products	2.1%	2.1%	0%	12.8%	83%
5. Fruits and their products	4.3%	2.1%	10.6%	36.2%	46.8%
6. Meat, poultry and their products	0%	0%	2.1%	4.3%	93.6%
7. Finfish, shellfish, other aquatic animals and their products	10.6%	10.6%	36.2%	34%	8.5%
8. Eggs	4.3%	4.3%	14.9%	36.2%	40.4%
9. Milk and its products	0%	12.8%	6.4%	23.4%	57.4%
10. Spices and condiments	6.4%	25.5%	19.1%	10.6%	38.3%
11. One-plate dishes, main dishes, local foods, and vegetarian foods	17%	17%	25.5%	25.5%	14.9%
12. Desserts and bakeries	19.1%	23.4%	31.9%	21.3%	4.3%
13. Insects/ Miscellaneous	95.7%	4.3%	0%	0%	0%

#### 4.3.2.3 Maternal nutrition status during the first month of lactation

Maternal nutrition status, including pre-pregnancy and current weight, weight gain during pregnancy, and body mass index (BMI), are displayed in Table 6. During the first month of lactation, most mothers (n=19, 40.4%) had a normal weight (18.5-24.9 kg/m<sup>2</sup>) or weights categorized as pre-obesity (25-29.9 kg/m<sup>2</sup>). The rest were reported as

obese ( $>30 \text{ kg/m}^2$ ) for 17% and underweight ( $<18.5 \text{ kg/m}^2$ ) for 2.1%, respectively. According to pre-pregnancy BMI, the majority of mothers ( $n=27$ , 57.4%) had a normal weight. While the rest of the mothers were overweight (25.5%), obese (10.6%), and underweight (6.4%), respectively. The median weight gain during pregnancy was 5.0 kg (3.6, 7.4). When comparing the difference in nutrition status between pre-pregnancy and current BMI, the Wilcoxon Signed Rank test indicated a significant difference between the median current BMI and pre-pregnancy BMI ( $Z = -5.790$ ,  $p < .001$ ). The median current BMI was significantly higher than the pre-pregnancy BMI ( $25.6 \text{ kg/m}^2$  vs.  $23.3 \text{ kg/m}^2$ ).

**Table 6** Nutrition status among Thai mothers of premature infants ( $n=47$ )

Parameter	Median (Interquartile Ranges)	Number (Percentage)
Pre-pregnancy weight (kg)	60 (55, 65)	
Current weight (kg)	65 (59, 73)	
Pre-pregnancy BMI ( $\text{kg/m}^2$ ) *	23.3 (20.0, 25.0)	
Current BMI ( $\text{kg/m}^2$ ) *	25.6 (23.0, 27.1)	
Weight gain during pregnancy (kg)	5.0 (3.6, 7.4)	
Pre-pregnancy nutritional status:		
• Underweight		3 (6.4%)
• Normal weight		27 (57.4%)
• Overweight		12 (25.5%)
• Obesity		5 (10.6%)
Current nutritional status:		
• Underweight		1(2.1%)
• Normal weight		19 (40.4%)
• Overweight		19 (40.4%)
• Obesity		8 (17%)

\*Statistically significant difference between current and pre-pregnancy BMI; Wilcoxon signed rank test:  $-5.790$ ,  $p < .001$ .

**4.3.3 Aim 3:** *To explore how maternal nutrition factors (current diet intake of macronutrient, habitual diet based on frequency of food consumption, current BMI), maternal demographic factors (maternal age, education level, multiple pregnancies, mode of delivery), and infant factors (gestational age and gender) can affect macronutrient components in mature milk.*

Several multiple regression models were constructed to assess the relationship between maternal factors, infant factors, and breast milk macronutrients. The independent variables in each model included current diet intake of macronutrients (daily total energy, carbohydrates, fats, and proteins intake), habitual diet (consumption frequency of various food categories), current BMI, maternal demographic factors (age, education level, multiple pregnancies, mode of delivery), and infant factors (gestational age and sex). Four models were developed to predict variation in each macronutrient in mature milk: BM fats (Model A), BM carbohydrates (Model B), BM energy (Model C), and BM proteins (Model D).

For model A (fats), the finding shows that mode of delivery ( $B = .847$ ,  $t = 2.926$ ,  $p = .006$ ), daily dietary of carbohydrates ( $B = .009$ ,  $t = 3.349$ ,  $p = .002$ ), fats ( $B = .013$ ,  $t = 1.977$ ,  $p = .045$ ), consumption frequency of cereal ( $B = 1.514$ ,  $t = 2.990$ ,  $p = .005$ ), and starchy roots ( $B = 2.373$ ,  $t = 2.936$ ,  $p = .006$ ) have significantly and positively correlated to breast milk fats. However, only the consumption frequency of dessert was significantly and negatively associated with breast milk fats ( $B = -.885$ ,  $t = -2.195$ ,  $p = .034$ ). Among these factors, daily dietary intake of carbohydrates had the most significant impact on increasing breast milk fats ( $\beta = 0.322$ ), followed by FFQ of cereal ( $\beta = .294$ ), FFQ of starchy roots ( $\beta = .293$ ), mode of delivery ( $\beta = .280$ ), and daily dietary of fats ( $\beta = .246$ ).

Conversely, only the FFQ of dessert significantly influenced the decrease in breast milk fats ( $\beta = -0.207$ ). The  $R^2$  value was .697, so 69.7% of the variation of breast milk fats can be explained by the model containing mode of delivery, daily dietary of carbohydrates, fats, and proteins, and consumption frequency of cereal, starchy roots, fish, and dessert.

For model B (carbohydrates), there was a significant and positive relationship between daily dietary intake of carbohydrates and breast milk carbohydrates ( $B = .006$ ,  $t = 2.877$ ,  $p = 0.006$ ) and between the frequency of consuming meat and carbohydrate levels in breast milk ( $B = 2.238$ ,  $t = 3.624$ ,  $p < 0.001$ ). FFQ of meats had the most significant impact on breast milk carbohydrates ( $\beta = .441$ ), followed by dietary intake of carbohydrates ( $\beta = .350$ ). The  $R^2$  was 0.360, meaning 36% of the variance in breast milk carbohydrates can be described by model B.

For model C (energy), significant positive associations were found between breast milk total energy and mode of delivery ( $B = 7.357$ ,  $t = 2.669$ ,  $p = .011$ ), daily dietary of carbohydrates ( $B = .107$ ,  $t = 4.336$ ,  $p < .001$ ), fats ( $B = .143$ ,  $t = 2.312$ ,  $p = .026$ ), proteins ( $B = .095$ ,  $t = 2.161$ ,  $p = .037$ ), and consumption frequency of starchy roots ( $B = 21.934$ ,  $t = 2.920$ ,  $p = .006$ ). Daily dietary intake of carbohydrates was the most significant predictor of breast milk total energy ( $\beta = .414$ ), followed by FFQ of starchy roots ( $\beta = .287$ ), daily dietary of fats ( $\beta = .282$ ), daily dietary of proteins ( $\beta = .259$ ), and mode of delivery ( $\beta = .255$ ), respectively. The  $R^2$  was 0.680, meaning 68% of the variance in breast milk total energy can be delineated by model C.

For model D (protein), three independent variables, including mode of delivery, daily dietary intake of fats, and frequency of consuming dessert, were significantly associated with breast milk protein levels. The mode of delivery and daily dietary intake

of fats significantly positively influenced on breast milk proteins ( $B = .230$ ,  $t = 2.125$ ,  $p < 0.040$  and  $B = 0.005$ ,  $t = 2.764$ ,  $p=0.009$ , respectively). In contrast, the frequency of consuming dessert significantly negatively impacted breast milk protein levels ( $B = -.530$ ,  $t = -3.473$ ,  $p=.001$ ). The frequency of consuming dessert had the most significant influence on decreasing breast milk proteins ( $\beta = -0.412$ ), while daily dietary intake of fats ( $\beta = 0.326$ ) had the most impact on increasing breast milk proteins, followed by mode of delivery ( $\beta = 0.250$ ). The  $R^2$  value for the model was 0.437, indicating that 43.7% of the variation in breast milk protein levels can be explained by model D.

**Table 7** Result of multiple linear regression analysis for exploring the impact of selected factors on breast milk macronutrient composition (n=47)

Selected variables	Unstandardized <i>B</i>	Coefficients Std. Error	$\beta$	<i>t</i>	<i>p-value</i>
Model A (Dependent variable: BM fats)					
Mode of delivery <sup>1</sup>	.857	.293	.280	2.926	.006**
Daily dietary of carbohydrates (g/day)	.009	.003	.322	3.349	.002**
Daily dietary of fats (g/day)	.013	.007	.246	1.977	.045**
Daily dietary of proteins (g/day)	.008	.005	.211	1.732	.091
FFQ of cereal and their product*	1.514	.506	.294	2.990	.005**
FFQ of starchy root and their product*	2.373	.808	.293	2.936	.006**
FFQ of fish*	.704	.378	.186	1.862	.070
FFQ of dessert*	-.885	.403	-.207	-2.195	.034**
R square .697					
Model B (Dependent variable: BM carbohydrates)					
Daily dietary of carbohydrates (g/day)	.006	.002	.350	2.877	.006**
FFQ of meat*	2.238	.617	.441	3.624	<.001**
R square .360					
Model C (Dependent variable: BM energy)					
Mode of delivery <sup>1</sup>	7.357	2.757	.255	2.669	.011*
Daily dietary of carbohydrates (g/day)	.107	.025	.414	4.336	<.001**
Daily dietary of fats (g/day)	.143	.062	.282	2.312	.026**
Daily dietary of proteins (g/day)	.095	.044	.259	2.161	.037**

<b>Selected variables</b>	<b>Unstandardized B</b>	<b>Coefficients Std. Error</b>	<b>β</b>	<b>t</b>	<b>p-value</b>
FFQ of cereal and their product*	8.910	4.613	.184	1.931	.061
FFQ of starchy root and their product*	21.934	7.513	.287	2.920	.006**
R square .680					
Model D (Dependent variable: BM protein)					
Mode of delivery <sup>1</sup>	.230	.108	.250	2.125	<.040**
Daily dietary of carbohydrates (g/day)	-.002	.001	-.230	-1.916	.062
Daily dietary of fats (g/day)	.005	.002	.326	2.764	<.009**
FFQ of meats*	.586	.305	.229	1.921	.062
FFQ of dessert*	-.530	.152	-.412	-3.473	.001**
R square .437					

\*Result from food frequency questionnaires; the response options were converted to continuous values as follows: "never" = 0, "less than once a week" = (0.5/7= 0.07), "Once or twice a week" = (1/7= 0.14), "more than twice a week but not every day" = (4.5/7=0.64), "every day" = 1.

\*\* Significant p-value less than .05; 1= mode of delivery; 0= vaginal delivery, 1= cesarean section

#### 4.4 Summary

This chapter provided findings on changes in breast milk macronutrient concentrations during the first month of lactation, maternal macronutrient intake, dietary habits, nutritional status, and the impact of maternal nutrition and demographic factors on macronutrient levels in breast milk. The findings revealed that breast milk carbohydrates and protein levels significantly changed from colostrum & transitional milk (during 1-2 weeks) to mature milk (during 3-4 weeks), while fat and energy levels did not differ significantly. Most mothers had lower daily energy intake than recommended for Thai people but higher intakes of fats, cholesterol, saturated fatty acids, proteins, sugar, and sodium. Almost half of mothers (40.4%) were currently overweight (BMI 25-29.9 kg/m<sup>2</sup>). For maternal dietary habits, the majority of mothers consumed a daily diet consisting of cereal (e.g., rice), vegetables, fruits, meat, eggs, and milk. Notably, only the mode of delivery in the demographic factors significantly influenced breast milk macronutrient levels. Also, maternal macronutrient intake, including daily intake of

carbohydrates, fats, and protein, were significantly associated with breast milk macronutrient concentrations.

## **CHAPTER 5**

### **DISCUSSION**

This study was conducted with forty-seven Thai mothers of premature infants to identify factors affecting macronutrient levels in breast milk by examining maternal nutrition and other relevant factors. To understand breast milk macronutrients and their association with maternal factors, a non-experimental correlational design was used. The findings show that some demographic and maternal nutrition factors were significantly associated with macronutrients in mature milk during the first month of lactation.

In this chapter, the researchers present synthesized study findings related to the study aims, examining their relevance to the existing literature and contributions to the current body of knowledge. It also discusses the study's strengths and limitations, as well as the implications of the findings for nursing practice and future research.

#### **5.1 Breast milk macronutrients at different stages of lactation (colostrum/transitional vs. mature milk)**

This study presented the macronutrient concentrations at different stages (colostrum & transitional vs. mature milk) during the first month of lactation among mothers of preterm infants. The findings revealed that protein and carbohydrate contents significantly differed between colostrum & transitional (1-2 weeks of lactation) and mature milk (3-4 weeks of lactation). As for protein, the concentration was high in the early period of lactation (colostrum & transitional milk) compared to the later period

(mature milk). In contrast, for carbohydrates, the concentration was low in colostrum & transitional milk compared to mature milk. Fat and energy levels in breast milk did not significantly differ during the first month of lactation.

The higher protein content during the colostrum & transitional phase in our study was consistent with many previous studies. During the early lactation period, the median value of protein content in preterm breast milk was 1.88 g/100 ml, and then it decreased during three to four weeks to 1.24 g/100ml (Boyce et al., 2016). This is consistent with studies of preterm infants where protein levels decreased after the first two weeks of lactation and then remained stable at the end of six weeks (Burianova et al., 2019; Saarela et al., 2005). Similarly, the results from a meta-analysis of macronutrient composition of preterm (<37 weeks gestation) breast milk showed that the mean protein levels during the first two weeks were higher (1.7 g/100ml) than protein levels during the three to four weeks of lactation (1.5 g/100ml) (Gidrewicz & Fenton, 2014). Our study confirms that the protein concentration in preterm breast milk is significantly associated with the lactation period, especially during the first month of lactation. This can be explained by the fact that colostrum & transitional milk, produced in low amounts during the first few days after delivery, contains a high concentration of immunological components such as lactoferrin, secretory IgA, and epidermal growth factor, which promote immune protection for preterm infants. Subsequently, the macronutrient composition of breast milk changes rapidly, with the protein concentration decreasing as the milk transitions to mature milk (more than 14 days of lactation) (Ballard & Morrow, 2013).

In contrast to protein, carbohydrate contents were reported as higher in mature milk. The median carbohydrate content in our study was 7.5 g/100ml for mature milk,

higher than 7.2 g/100ml for colostrum & transitional milk. This finding aligns with a recent study from the Czech Republic, where the median carbohydrate content was 6.9 g/100ml in mature milk and 6.5 g/100ml in colostrum & transitional milk (Burianova et al., 2019). A systematic review of preterm breast milk composition also supports this trend, with carbohydrate content increasing from 6.55 g/100ml in colostrum & transitional milk to 7.28 g/100ml in mature milk (Boyce et al., 2016). Mature milk, produced after the first two weeks of lactation, contains higher carbohydrate and fat levels to meet the nutritional needs of preterm infants. The higher carbohydrate concentration in mature milk serves as a primary energy source for preterm growth and development (Ballard & Morrow, 2013) and plays a crucial role in maintaining the composition of intestinal microbiota in preterm infants (Boudry et al., 2021). Generally, mature breast milk contains around 7% carbohydrates, primarily lactose (70%) and oligosaccharides (30%) (Kim & Yi, 2020). In our study, we focused on overall carbohydrate values instead of lactose or oligosaccharides to capture all carbohydrate contents in preterm breast milk.

The median value of fat content in our study was increased from 3.8 g/100ml during the colostrum & transitional milk to 4.2 g/100ml in mature milk with no statistically significant difference during the first month of lactation. This aligns with results from a previous systematic review of preterm breast milk composition. The breast milk fat content was changed from 2.63 g/100ml during the first week of lactation to 3.54 g/100ml after two weeks of lactation (Boyce et al., 2016). Likewise, the longitudinal study of preterm breast milk reported the fat concentration increased during the mature milk when compared to colostrum milk and then gradually changed stable concentration

after 5 weeks to maintain a level to support preterm nutrition needs (Bauer & Gerss, 2011). The high fat content during the mature milk in the first month is biologically change to response for the higher demand for preterm growth and development. Fat in breast milk plays an important role in the energy supply for preterm infants and the central nervous system development (Kim & Yi, 2020). Fat is also known to be the most variable macronutrient in breast milk. It has been reported that fat levels vary depending on maternal nutrition factors (Ballard & Morrow, 2013). This is a possible reason to explain the finding that our fat content increased during mature milk, but there was no significant statistical difference.

The median of energy content in our study increased from 72.7 kcal/100ml during the colostrum & transitional milk to 76.7 kcal/100ml in the mature milk, with no significant statistical difference. This is congruent with a meta-analysis of preterm breast milk from Canada involving 843 mothers of preterm infants, where the average energy content increased from 71 kcal/100ml during the colostrum & transitional milk to 77 kcal/100ml in the mature milk (Gidrewicz & Fenton, 2014). Similarly, a study conducted in the United States showed that the average of energy content in breast milk was higher in mature milk (70.4kcal/100ml) than in colostrum milk (69.9 kcal/100ml) (Gates et al., 2021). This can be explained by the fact that the total energy content in breast milk has a positive relationship with the fat and carbohydrate content (Bauer & Gerss, 2011). Carbohydrates and fat contribute 40% and 50%, respectively, to the total energy value of breast milk (Martin et al., 2016). The increased carbohydrate and fat content in mature milk contributes to the higher energy content compared to colostrum milk. Additionally, the energy content of breast milk varies depending on the breast milk fat, which is the

macronutrient most highly affected by maternal nutritional factors (Ballard & Morrow, 2013). This could result from increased energy content in mature breast milk, but no significant difference exists in this study.

## **5.2 Daily energy and macronutrient intake of mothers of preterm infants in Thailand**

This study presents the average daily energy and macronutrient intake among Thai mothers of premature infants during the first month of lactation. The findings reveal that most mothers had lower energy intake than the recommended daily allowance (RDA) for lactating Thais and had higher intakes of fat, cholesterol, saturated fatty acids, protein, sodium, and sugar than recommended.

The average energy intake among mothers (2,123 kcal/day) was lower than the recommended level for lactating women. According to the Dietary Reference Intake for Thais 2020 (Thai DRI) developed by the Bureau of Nutrition, Department of Health, Ministry of Public Health, Thailand, lactating women should consume an additional 500 kcal/day during the first 6 months of lactation to support their breast milk production (Bureau of Nutrition, Department of Health, Ministry of Public Health, 2020). The higher energy demand during lactation is derived from the mean breast milk volume produced per day (mean 780 ml) and the energy content of breast milk (67 kcal/100 ml) (Kominiarek & Rajan, 2016). Therefore, the average energy intake required for lactating mothers in our study should be approximately 2,280 kcal/day, compared to non-lactating women aged between 19-50 years, who require 1,780 kcal/day. Our finding was similar to recent data from a study among lactating women in Southern Thailand. The average

energy intake was 1,865 kcal/day, lower than the recommendation (Puwanant et al., 2022). It could be explained that the lower energy intake may result from cultural dietary habits in Thailand. Thai meals are typically served family-style, with multiple dishes shared among everyone at the table. This communal eating style often results in smaller portion sizes and lower overall daily energy intake (Bureau of Nutrition, Department of Health, Ministry of Public Health, 2020). Another reason could be the common problem of people tending to underreport their dietary intake in 24-hour dietary recall records (Ivanovitch et al., 2014). Underreporting energy intake is a well-known issue in self-reported dietary assessments across various developed and developing countries (Scagliusi et al., 2006). In this study, the Goldberg cut-off 1 test, which identifies under-reporters and over-reporters in dietary records, revealed that nine mothers (19%) underreported their daily energy intake, as indicated by an energy intake (EI) to basal metabolic rate (BMR) ratio of less than 1.35 (Goldberg et al., 1991). This finding is consistent with another study showing that Thai women aged 20-50 underreported energy intake by 11% (Ivanovitch et al., 2014). Further studies are needed to minimize this issue by using multiple food dietary records and technology-assisted tools to capture more accurate nutrient intake reports.

The average daily intake of fat, cholesterol, and saturated fatty acids were also reported as higher than recommended among mothers in this study. The mothers had a higher average fat intake of 91 g/day (38% of total energy) instead of the recommended 78 g/day (20-35% of total energy). Cholesterol intake among mothers was almost twice as high as the recommended 558 mg/day instead of 300 mg/day. Saturated fatty acid intake was higher at 26 g/day instead of the recommended 20 g/day. These results were

consistent with the fourth National Health Examination Survey in 2009, presenting that fat consumption increased from 8.9% of total energy intake in 1960 to 28% in 2009 among Thai adult population, and the trend is more likely to increase in the next 10 years (Aekplakorn et al., 2009). A study conducted in lactating women from Southern Thailand also supports this finding where the mean cholesterol intake of lactating women (387 g/day) was higher than the recommendation (Puwanant et al., 2022). This could be the result of shifting dietary consumption patterns from a traditional Thai diet high in starchy staples and vegetables to a more Westernized diet high in fat and ultra-processed foods (Phulkerd et al., 2023).

The average daily intake of protein found in this study was higher (117 g/day or 21% of total energy intake) than those recommended by Thai DRI (72g/day or 10 to 15% of total energy intake). Lactating women should intake additional protein of 19 g/day, requiring a total protein intake of 72 g/day during the first 6 months of lactation to maintain their energy in milk production when compared to non-lactating women aged 19-50 years who require protein intake of 53 g/day (Bureau of Nutrition, Department of Health, Ministry of Public Health, 2020). The higher protein intake was similar to the data report in Thai nutrition survey, showing that the protein intake among Thai people increased from 10% of total energy in 1960 to 16% of total energy in 2009 (Aekplakorn et al., 2011; Kosulwat, 2002). This is another confirmation of how much the traditional Thai diet based on carbohydrates has changed to modern nutrition patterns that contain a higher proportion of fats and protein-rich food groups (e.g., animal meat) (Kosulwat et al., 2002; Phulkerd et al., 2023).

Our study also revealed that mothers of preterm infants had a higher daily sodium intake (3,518 mg/day) than the recommended amount according to the Thai DRI, which should not exceed 1,550 mg/day (Bureau of Nutrition, Department of Health, Ministry of Public Health, 2020). This finding confirms the high sodium consumption among Thai people, consistent with many other studies conducted in Thailand. According to the Thai National Survey in 2020, the mean dietary sodium intake in the Thai population in 2019-2020 was 3,636 mg/day, which is twice the recommended amount by the Thai DRI (Chailimpamontree et al., 2021). The high sodium intake among Thai people can be attributed to the common use of condiments and seasonings in cooking and at the table. Many of these condiments, such as fish sauce, oyster sauce, soy sauce, salt, and seasoning powder, contain high levels of sodium (Ivanovitch et al., 2014). Additionally, monosodium glutamate (MSG) is frequently used in Thai recipes for its umami flavor (Lioe et al., 2005). Although condiments with alternative sodium, such as less sodium sauces or no added MSG condiments, are available in many supermarkets in Thailand, these condiments are not popular among Thai people due to the higher price (Jindarattanaporn et al., 2023). Another contributing factor is the increased consumption of ready-to-eat foods, such as instant noodles, frozen foods, and snacks, typically high in sodium (Phulkerd et al., 2023). Excessive sodium intake remains a global issue. Reports from Western countries indicate similarly high average sodium intakes: the United States at 3,608 mg/day, Canada at 3,325 mg/day, and Australia at 3,325 mg/day (Huang et al., 2016). High sodium intake is associated with various health problems, including hypertension, chronic kidney disease, and cardiovascular disease (He & MacGregor, 2009). Thailand has set a goal to reduce population sodium intake by 30%, in line with

the World Health Organization's (WHO) global targets for a 30% relative reduction in mean population sodium intake by 2025 (Ministry of Public Health of Thailand, 2022). However, our findings suggest that there is a need for more effective public health campaigns and national health policies to promote lower sodium consumption among the Thai population.

High intake of sugar was also reported in this study. All mothers in our study (100%, n=47) had a daily sugar intake higher than the recommended amount by the Thai Dietary Reference Intake (DRI), with a mean of 73 g/day compared to the DRI recommendation of 24 g/day. This finding is consistent with a review paper examining sugar consumption levels in the Thai population, which showed a higher consumption of approximately 83 g/day among Thai adults (Kriengsinyos et al., 2018). The higher sugar intake is not surprising, as the National Food Consumption Behavior Survey in 2017 reported that over 50% of Thai people often consumed sugar-sweetened beverages (National Statistical Office, 2018). The higher intake of sugar could be the result of the increased consumption of ready-to-eat foods, which are pre-prepared and can be consumed immediately without any further preparation, among the Thai population (National Statistical Office, 2018). Most ready-to-eat foods are ultra-processed, involving multiple processes with the addition of salt, unhealthy fats, artificial flavors, or sugar, resulting in a poor nutritional profile and high levels of sugar (Phulkerd et al., 2023). Higher intake of sugar is significantly correlated with many health problems, such as diabetes, dyslipidemia, and hypertension (Mojto et al., 2019). Our study supports that higher consumption of sugar intake remains an important health issue in Thailand.

### 5.3 Maternal Dietary Habits

Cereals, mainly rice, vegetables, fruits, meat, eggs, milks, spices, and condiments, were reported as food groups consumed daily by most mothers in this study. Our finding is consistent with many previous studies examining the Thai population's dietary habits. The study conducted among lactating women from Southern Thailand also reported that the common food groups consumed by lactating women were rice, vegetables, fruits, meat, and eggs (Puwanant et al., 2022). Similar to the study by Papier and colleagues (2017), vegetables and white rice were the most commonly consumed foods among Thai adults. This could be due to the fact that the origins of the Thai diet can be traced back to water-borne communities. King Ramkhamhaeng's famous stone inscription from the early 13th century reveals that rice and fish were the primary ingredients in Thai cuisine. Vegetables, fruits, and herbs and condiments were common basic ingredients in Thai foods (Kosulwat, 2002). This traditional Thai food continues to influence the dietary habits of Thai people today, with their diets consisting of approximately 70-80% carbohydrates, primarily from jasmine or sticky rice and many condiments and spices (Kriengsinyos et al., 2018). However, development and urbanization have resulted in the shift of food diet habits from traditional diets toward diets containing higher proportions of fats, animal meat, dairy products (e.g., milk), and eggs (Kosulwat, 2002). Also, a shift in diet habits from preparing food at home to purchasing one-plate dishes or ready-to-eat foods are reported among Thai people due to social and economic changes (Papier et al., 2017). In this study, we found that more than 50% of mothers consumed one-plate dishes once or twice a week up to every day. In this study, a one-plate dish refers to a cooked-to-order meal that is served and eaten from a single plate. These dishes typically include a

mix of carbohydrates, proteins, and vegetables, providing a convenient, all-in-one meal that requires minimal preparation and cooking time, such as Khao Pad Ka Pao (rice with stir-fried basil with meat), Pad Thai, Tom Yum Fried Rice, etc. Most one-plate dishes can be purchased from many restaurants in Thailand (Judprasong et al., 2018). The finding was similar to the National Household Economic Survey, which showed that almost 50% of the expenditure on food was for purchasing ready-to-eat food rather than food prepared at home (Kosulwat, 2002). It has been speculated that the economic growth and changes to industrial society during the past 50 years in Thailand have transformed people's lives to lifestyles that require less time and skill to prepare food. Home-made foods are not popular and are being replaced by purchased food bought from outside homes like one-plated dish (National Statistical Office, 2017). Another possible reason is that most mothers need time to care for their infants. Therefore, they prefer to eat one-plate dishes that require less preparation time rather than cooking meals at home. Moreover, the consumption of pulses, seeds, and nuts is lower among the mothers in this study. Most of them (76.6%) reported consuming these foods only once or twice a week, or not at all. This is not surprising, as most Thai people consume nuts as snacks rather than daily. Additionally, Thai cuisine typically does not include nuts as a common ingredient (Puwanant et al., 2022). This may lead to insufficient intake of certain nutrients, such as healthy fatty acids, vitamins, and minerals, which are naturally sourced from this food group. For desserts and bakery items, this study presented that the majority of mothers (74.4%) reported consuming desserts once or twice a week or not at all. Although the Thai cultural diet commonly includes consuming dessert after the main meal (Jindarattanaporn et al., 2023), some participants indicated that they were more

likely to decrease their consumption of desserts high in unhealthy fats to prevent negative outcomes on their health and the nutritional content of their breast milk for their infants. This finding confirms the dietary habits related to health concerns, especially regarding desserts, among mothers of preterm infants. This is consistent with the consumption of insects, which are not typically part of the diet among mothers of preterm infants.

#### **5.4 Maternal nutrition status during the first month of lactation**

The median of current BMI during the first month of lactation was higher than pre-pregnancy BMI among mothers in our study (25.6 kg/m<sup>2</sup> vs. 23.3 kg/m<sup>2</sup>;  $p < .001$ ). This phenomenon is commonly observed in women worldwide. During pregnancy, pregnant women need additional energy for tissue building and fetal growth, resulting in the need for weight gain during pregnancy (Bureau of Nutrition, Department of Health, Ministry of Public Health, 2020). According to the Institute of Medicine and National Research Council (2009), the appropriate gestational weight gain for a pregnant woman with normal pre-pregnancy BMI (18.5-24.9 kg/m<sup>2</sup>) is approximately 12 kilograms throughout the pregnancy, with varying weight gains in each trimester. During the first trimester, mothers should have an average weight gain of 1 kilogram, with the remaining weight gain occurring at a rate of 0.4-0.45 kilograms per week during the last two trimesters. However, the median weight gain among our participants was 5 kilograms lower than this recommendation. This can be explained by the fact that our study was conducted among mothers who had delivered preterm infants. These mothers may have experienced reduced weight gain during the third trimester, resulting in lower overall

weight gain compared to mothers who delivered full-term infants with full weight gain during the third trimester.

Considering the current BMI during the first month of lactation, the majority of mothers were overweight (40.4%) and obese (17%). Postpartum weight retention can shift women from a normal weight BMI classification before pregnancy to an overweight classification postpartum (US Department of Agriculture and US Department of Health and Human Services, 2020). Generally, postpartum weight retention can take six months to a year to return to pre-pregnancy weight (Makama et al., 2021). However, our study is limited as it only covers the first month of lactation. Therefore, we cannot fully classify maternal BMI based on their weight gain during pregnancy, resulting in the observed high BMI during the first month of lactation.

## **5.5 Macronutrients in breast milk and their association with relevant factors**

### **5.5.1 Macronutrient composition in breast milk in relation to maternal nutrition factors**

Our study confirms that macronutrient composition in breast milk can be influenced by some maternal nutrition factors, including dietary intake of carbohydrates, protein, and fat, as well as consumption frequency of cereal, starchy roots, meat, and dessert. However, the study did not find any association between maternal BMI and macronutrients in breast milk.

The role of maternal diet intake (daily dietary intake of macronutrients and frequency of food consumption) as a determinant of macronutrients in breast milk has been examined in many studies for decades with inconsistent results (Adhikari et al.,

2021; Bravi et al., 2016). Breast milk fat is reported as the most common macronutrient that appears to be strongly influenced by dietary intake from mothers (Adhikari et al., 2021). The finding of our study supports these findings by showing that maternal diet intakes, including daily dietary of carbohydrates, fats, consumption frequency of cereal, and starchy roots, are significantly and positively correlated with breast milk fat content, while frequent dessert consumption is significantly and negatively associated with breast milk fat levels. This was similar to previous studies conducted among mothers of infants under 6 months of age (Hascoet et al., 2019; Kim et al., 2017). A study from France showed that dietary intake of carbohydrates among mothers was positively correlated to fat levels in their breast milk (Hascoet et al., 2019). Another study from Korea also found that dietary intake of fat among Korean mothers was positively associated with fat levels in breast milk (Kim et al., 2017). This could be due to the fact that breast milk fats are derived from a combination of maternal nutrient stores and dietary intake (Ward et al., 2021). The high intake of fat and carbohydrates among mothers can stimulate lipogenesis and contribute to higher fat content in their breast milk (Ross et al., 2024). Also, the frequency of cereal and starchy roots can relate to breast milk fat content due to higher rich sources of carbohydrate, which can influence fat in breast milk (Huang & Hu, 2020). However, the negative association between the frequency of dessert consumption and breast milk fat levels was unexpected. This may be due to the fact that most desserts contain higher amounts of sugar, which can disrupt the maternal metabolic process, leading to alterations in the composition and amount of fat in breast milk.

Similar to breast milk fat, breast milk energy levels are mostly associated with the mother's dietary intake (Adhikari et al., 2021). A previous systematic review exploring

the influence of maternal diet on breast milk macronutrient composition revealed that maternal dietary intake of carbohydrates, fat, and protein had a significant association with total breast milk energy (Adhikari et al., 2021). This is congruent with our study findings that dietary intake of carbohydrates, fat, and protein, as well as consumption frequency of starchy roots, is significantly associated with total breast milk energy.

For the breast milk carbohydrate levels, which is reported as the least variable of the macronutrients (Petersohn et al., 2024). The consistency of carbohydrate levels is crucial for maintaining a constant osmotic pressure in breast milk (Bzikowska-Jura et al., 2018b). The osmotic pressure of milk is directly proportional to the number of particles in solution of breast milk. Generally, carbohydrates secreted by mammary epithelial cells accounts for over 50% of breast milk's osmotic pressure. Increased carbohydrates synthesis in the mammary gland draws more water from the blood into the gland's lumen, maintaining stable osmotic pressure in breast milk (Silanikove et al., 2006). This constant carbohydrate levels helps balance the concentration of nutrients and fluids in the breast milk (Martin et al., 2016). However, previous studies reported that maternal dietary intake of some macronutrients such as carbohydrates and protein may influence on breast milk carbohydrates. According to a cross-over study from the United States, maternal intake of high carbohydrates had higher carbohydrates in breast milk compared to those who did not consume high carbohydrates (Berger et al., 2018). Another study from Japan found that during the first month of lactation, only maternal diet intake of protein was positively correlated to breast milk carbohydrates (Minato et al., 2019). These findings are similar to our study that found some aspects of maternal dietary intake, including dietary intake of carbohydrates and frequency of consuming meat, had a significant and

positive association with carbohydrate levels in breast milk. In contrast, several studies support the opposite results that carbohydrates in breast milk are not influenced by maternal dietary intake (Aumeistere et al., 2019; Bzikowska-Jura et al., 2018b). This discrepancy may be due to the fact that our study was conducted during the first month of lactation, providing a much greater change in carbohydrate content over the lactation stage (from colostrum to mature milk). As a result, the carbohydrate content can be more influenced by maternal diet than later periods when carbohydrate levels remain constant to maintain osmotic pressure in breast milk. Therefore, the role of maternal diet on the variation of carbohydrates needs to be further explored.

In terms of breast milk protein concentration, this study showed that some macronutrient intake, such as fat, was positively correlated to breast milk proteins, while the frequency of dessert consumption was negatively correlated to protein levels in breast milk. This could be the result of the need for a higher energy supply for the synthesis of protein in breast milk (Bionas et al., 2012). Maternal diet of fat provides higher energy availability to contribute to breast milk protein among mothers (Ward et al., 2021). However, the negative association between the frequency of dessert consumption and breast milk protein levels was also reported in our study. As mentioned above, desserts contain higher amounts of sugar, which can disrupt the maternal metabolic process, leading to alterations in any breast milk macronutrient. These metabolic processes are not well understood and need further study.

In brief, a potential influence of maternal diet intake on breast milk macronutrients mainly occurs in breast milk fat and energy contents compared to breast milk carbohydrates and proteins. Specifically, although some macronutrient diet intakes

were reported to be related to breast milk carbohydrates and proteins, this remains the inconclusive result of maternal diet and their association with breast milk carbohydrates and proteins. One possible reason is that the limited time conducted during this study (only the first month of lactation) may potentially affect the association between maternal diet intake and breast milk nutrients under study. There is a need for longitudinal study to examine the role of maternal diet intake and breast milk nutrients, especially protein and carbohydrate, among Thai mothers.

For maternal BMI, we found no evidence of an association between maternal BMI during the first month of lactation and any breast milk macronutrient compositions. This finding is consistent with previous studies, which revealed that breast milk macronutrients, including carbohydrates, fat, and protein, were not significantly influenced by maternal BMI and weight during breastfeeding (Antonakou et al., 2013; De Luca et al., 2016). In contrast, a study conducted in Poland found that maternal BMI was positively correlated with fat levels in mature milk during the first month of lactation (Bzikowska-Jura et al., 2018b). Similarly, a meta-analysis investigating the effect of maternal nutritional status on macronutrients in breast milk indicated that maternal BMI, especially overweight and obesity, was associated with higher fat and carbohydrate content in breast milk (Leghi et al., 2020). Although many past studies reported a relationship between maternal BMI and some breast milk macronutrients, such as fat and carbohydrates, these associations were often limited to obese women (BMI > 30 kg/m<sup>2</sup>). It is possible that the metabolic imbalances frequently observed in women with obesity, specifically dyslipidemia and elevated circulating triglyceride levels, may be linked to increased fat content in breast milk (Leghi et al., 2020). In our study, the median current

BMI among mothers was reported as 25.6 kg/m<sup>2</sup>, which is not high enough to be considered obese and affects breast milk macronutrients through metabolic regulation. Additionally, it should be noted that increasing maternal adiposity (body fat mass) is more likely to be strongly correlated with breast milk macronutrients than BMI (Bzikowska-Jura et al., 2018b). Women with high fat mass have been reported to have impaired milk sugar synthesis, increased fat content in breast milk due to metabolic dysregulation, and elevated protein in breast milk according to increasing serum amino acids (Kuganathan et al., 2017; Leghi et al., 2020). In this study, we did not evaluate body compositions among mothers and only measured BMI. BMI is not a direct measurement of adiposity (body fat), so further studies need to explore how maternal nutritional status, including body fat mass, muscle mass, or other components, can affect breast milk nutrients among Thai mothers of preterm infants.

### **5.5.2 Macronutrient composition in breast milk in relation to maternal demographic and infant factors**

We analyzed various maternal and infant factors, including maternal age, education level, multiple pregnancies, mode of delivery, gestational age, and infant gender, and their association with breast milk macronutrients. Our study found that the mode of delivery was the only significant predictor associated with breast milk fats, energy, and protein content. Mothers who had cesarean sections showed higher levels of fat, energy, and protein in their breast milk compared to those who had vaginal deliveries. This finding aligns with Burianova et al. (2019), who examined maternal characteristics and their association with breast milk nutrients among mothers of preterm infants. The study reported that the mode of delivery significantly influenced breast milk protein

content for the first six weeks after delivery, with cesarean sections resulting in higher protein content than vaginal deliveries. A study from Turkey (Dizdar et al., 2014) also supports this finding, showing that the mode of delivery was associated with protein levels in breast milk. It has been speculated that mothers of preterm infants often undergo emergency cesarean sections due to health conditions. This sudden change from the expected vaginal delivery to cesarean section might impact hormones that affect breast milk nutrients (Burianova et al., 2019).

Other factors, including maternal age, education level, multiple pregnancies, gestational age, and infant gender, were reported as no association with breast milk nutrients in many past studies. Hascoet et al. (2019), determining the effect of maternal factors on breast milk macronutrients during the first five weeks of lactation, found no relationship between milk nutrient composition and mother's age, multiple pregnancies, and gestational age at delivery. Burianova et al. (2019) similarly showed no association between maternal age and breast milk nutrients. Thakur et al. (2021) also supported the finding that macronutrients in breast milk were independent of multiple pregnancies and gestational age. This is consistent with study by Maly et al. (2019), which evaluated macronutrient levels in breast milk at different gestational ages (24-30 weeks vs. 31-35 weeks) and reported that breast milk nutrients were not influenced by gestational age. Furthermore, a previous observational study by Mangel et al. (2020) confirmed that there were no significant differences in breast milk macronutrients between female and male infants (Mangel et al., 2020).

## 5.6 Strengths and Limitations

This study provides a comprehensive assessment of breast milk macronutrients during the first month of lactation and their association with maternal nutrition, demographics, and infant factors among Thai mothers of premature infants. The strengths of this study include the meticulous process of milk collection and analysis procedures, which minimized potential errors in measuring macronutrients in breast milk.

Researchers provided professional support to each participant, instructing them on proper milk sampling techniques prior to collection. The sampling process was standardized by collecting milk at a specific time (7:00 AM - 11:00 AM) using only electric breast pumps, with participants expressing milk until one entire breast was emptied. This approach helped minimize the potential influence of circadian rhythms and collection methods on breast milk macronutrient composition. Furthermore, milk samples were analyzed using fresh or refrigerated milk rather than frozen milk, avoiding any potential effects of freezing and rewarming on breast milk composition. Additional strengths of this study include using two measurement methods (24-hour food recall and food frequency questionnaire) to assess maternal diet, as well as using the Thai software program INMUCAL, which is the standard program for the accurate calculation of nutrients in specific Thai food.

However, the present study has some limitations. Firstly, the research was conducted with a specific group of Thai mothers of premature infants, and therefore, the results should not be generalized to other ethnic groups. Secondly, the study only covered the first month of lactation, highlighting the need for longitudinal research to examine how maternal and infant factors impact breast milk nutrients over an extended lactation

period. Additionally, in assessing maternal nutritional status, this study did not employ other anthropometric methods, such as measuring fat mass or muscle mass, which may influence breast milk macronutrient composition. Finally, although we used two measurement methods to assess maternal diet, the 24-hour food recall record may be subject to bias as it relies on participants self-reporting their intake, potentially leading to under- or over-reporting of actual consumption. Currently, there is no gold standard method for preventing inaccurate reporting of nutritional intake. In our study, we aimed to minimize this bias by using household measurements familiar to participants for reporting portion sizes, and by confirming food intake with pictures provided by participants to obtain more accurate data. However, further studies are needed to explore other methods, such as mobile applications, to record diet intake precisely.

## **5.7 Conclusion and Implications**

In conclusion, this study has demonstrated that breast milk macronutrients vary significantly during the first month of lactation, particularly in terms of protein and carbohydrate concentrations. Our findings indicate that protein levels tend to decrease from colostrum to mature milk. This declining protein content may not be sufficient to meet the nutritional needs of preterm infants. Consequently, our study supports the use of protein fortification in breast milk, especially during the mature milk phase, to enhance its nutritional value. Furthermore, our research suggests that breast milk macronutrients should be regularly measured to enable individualized fortification, ensuring appropriate growth and development of preterm infants. We also found that maternal nutritional factors, such as dietary intake of carbohydrates, fats, and proteins, as well as the

frequency of consumption of cereals, starchy foods, meat, and desserts, significantly influence macronutrient levels in breast milk. Therefore, monitoring these factors and understanding their association with breast milk macronutrients should be considered. Although our study revealed correlations between some maternal nutrition and breast milk composition, further research is needed to confirm these findings. Future studies should employ a longitudinal design and utilize multiple accurate measurements of maternal diet and nutritional status. Moreover, they should explore a broader range of breast milk components among Thai mothers of preterm infants, including macronutrients and micronutrients.

**APPENDIX A**  
**24 HOURS-FOOD RECALL RECORD**

## 24-hours food recall record

### Instructions on form completion

- A 24-hours food recall record aims to ask lactating women for their recalling and describing all foods and beverages consumed in the previous 24 hours.
- A recall interview typically conducts by line application and requires 20 to 30 minutes to complete, but it may take considerably longer depending on different food and drink intake.
- The form will be started with asking all the list of foods and drinks consumed by beginning first thing eaten in the morning until the last food item consumed before waking up the next morning.
- Next, probe questions about foods and drinks including **Beverages, Alcoholic beverages, Sweets, Snacks, Fruits, vegetables, Breads** and **Anything else/condiments** will be conducted to help the respondent remember foods in case of they forgot, especially if very few foods and drinks have been reported.
- Then, list the approximate **Time** the meal was consumed, **Place** where it was consumed (home, work, name of restaurant, market etc.) and type of eating occasion or **Meal** (breakfast, lunch, dinner, snack, or other).
- Record for each food/beverage consumed: **name of food, food description, household measure** (e.g. rice serving spoon, cups, dishes, tablespoons or teaspoons etc.), **unit of measure if possible** (e.g. gram, oz. and ml etc.), finally the kind of **preparation methods used and/or ingredients** (e.g. sugar, oil).
- \*Record the amount of each food/beverage, the portion size will record using standard measurement and household utensil
  - Weight in grams or ounces/ml
  - Solid foods-use volume in rice serving spoon, cups, dishes, tablespoons or teaspoons
  - Liquids-use volume in fluid ounces or ml

- In order that the record is as accurate as possible, probe further at the end of the interview for anything else consumed such as water and review accurate information with the respondent will be performed before complete form.

**Participant Number:** \_\_\_\_\_

**Date of recall:** \_\_\_ / \_\_\_ / \_\_\_

**Previous day:** 1. Weekday 2. Weekend day or public holiday \_\_\_ / \_\_\_ / \_\_\_

<b>Quick list Food/beverage name</b>	<b>Food/beverage description</b>	<b>Time</b>	<b>Place</b>	<b>Meal</b>	<b>Household amount</b>	<b>Amount (g/ml/oz)</b>	<b>Preparation/ingredients</b>

**APPENDIX B**  
**FOOD FREQUENCY QUESTIONNAIRES**

## Food Frequency Questionnaires (FFQs)

### Instructions on form completion

- A FFQs form used to evaluate habitual diet by asking about the frequency with food items are consumed in a group of lactating women who had premature infant in Thailand.
- This food frequency questionnaires will recall food that lactating women ate during the one month of lactation period.
- First, fill out below the personal information (participant ID number and date etc.)
- Mark with tick (✓) on the frequency that lactating women consumed the food record: “never”, “less than once a week”, “once or twice a week”, “more than twice a week but not every day”, “everyday”

**Participant Number:** \_\_\_\_\_

**Date of recall:** \_\_\_\_/\_\_\_\_/\_\_\_\_

Food items	Average use past month				
	Never	Less than once a week	Once or twice a week	More than twice a week but not every day	Everyday
1. Cereals and their products e.g. rice, bread					
2. Starchy roots, tubers and their product					
3. Pulses, seeds, nuts and their products					

Food items	Average use past month				
	Never	Less than once a week	Once or twice a week	More than twice a week but not every day	Everyday
4. Vegetables and their products					
5. Fruits and their products					
6. Meat, poultry and their products					
7. Finfish, shellfish, other aquatic animals and their products					
8. Eggs					
9. Milk and its products					
10. Spices and condiments					
11. One plate dishes, main dishes, local foods and vegetarian foods					
12. Desserts and bakeries					
13. Insects/ Miscellaneous					

## APPENDIX C

### MATERNAL BODY MASS INDEX (BMI) FORM

#### Instruction

The maternal body mass index (BMI) form is completed during the 2<sup>nd</sup> timeline of study session (between 3-4 weeks of lactation) to record maternal anthropometric results. To control quality of measurement, the scale is calibrated daily. For weight, it is measure using standing digital weighing scale and should be taken with minimal clothing. Record weight to the tenths place (one decimal) with the kilogram (kg) unit. For height, it is measure with portable stadiometer and record to the tenths place (one decimal) with the centimeter (cm) unit. All weight and height should be done twice time. Then, weight and height will be calculated to the body mass index (BMI) which was calculated by squaring the height in meters and then dividing the weight by height in meters squared (kg/m<sup>2</sup>).

**Participant Number:** \_\_\_\_\_ **Date of record:** \_\_\_\_/\_\_\_\_/\_\_\_\_

Pre-Pregnancy weight \_\_\_\_\_ kg

Weight: 1<sup>st</sup> \_\_\_\_\_ kg      2<sup>nd</sup> \_\_\_\_\_ kg

: Average weight \_\_\_\_\_ kg

Height: 1<sup>st</sup> \_\_\_\_\_ cm      2<sup>nd</sup> \_\_\_\_\_ cm

: Average height \_\_\_\_\_ kg

Maternal body mass index (BMI): \_\_\_\_\_

Nutrition status based on BMI following WHO recommendation

underweight: < 18.5

normal weight: 18.5-24.9

Pre-obesity: 25.0-29.9

Obesity:  $\geq$  30

## APPENDIX D

### DEMOGRAPHIC DATA RECORD

**Instruction:** This record form aims to collect socio-demographic data of mothers who give premature birth. The researcher record and mark (✓) on the information that match the truth

**Participant Number:** \_\_\_\_\_ **Date of record:** \_\_\_\_/\_\_\_\_/\_\_\_\_

**Age:** \_\_\_\_\_ years

**Education level:**

Lower than High School

High School Graduate

Bachelor's degree

Beyond Bachelor's degree

**Parity:** \_\_\_\_\_

**Multiple Pregnancies:**  No  Yes, specify (twin, triplet, etc.) \_\_\_\_\_

**Mode of delivery:**

Vaginal delivery

Cesarean birth

**Infant gender**  Male

Female

**Gestational age at birth:** \_\_\_\_\_ weeks

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