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The actual zinc and iron intakes among breastfed infants during the first 4 months of life

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Key Words

Zinc deficiency, iron deficiency, breastfeeding, breast milk zinc concentration, breast milk iron concentration, zinc intake, iron intake

Abstract

Background: During exclusive breastfeeding period, breast milk is only source for zinc and iron intakes of breastfed infants. The adequacy of zinc and iron intakes should be ensured to prevent deficiencies in breastfed infants.

Method: A longitudinal study followed pregnant women to lactation period. Maternal nutrient intakes were assessed during pregnancy and lactation. Anthropometric assessment of mothers and infants were performed. To estimate nutrient intakes, zinc and iron concentration in breast milk were determined at 2 and 4 months postpartum, together with assessment of the breast milk volumes. Adequacy of zinc and iron intakes were determined comparison to average requirements. Cord blood zinc and ferritin were determined as nutrient stores at birth. Maternal and infant serum zinc and ferritin were analysed at 4 months postpartum.

Results: 120 mothers were enrolled, and 56 mother-infant pairs who continue breastfeeding to 4 months postpartum. Pre-pregnancy BMI and mode of delivery were associated with cord blood zinc and ferritin level. Zinc intake from breast milk significantly decreased from 2 to 4 months. Inadequacy of zinc intake was found in 14.5% and 40% of infants aged 2 and 4 months, respectively. Iron intake was adequate. Maternal dietary intakes and anthropometric measurement were not associated with breast milk nutrient concentrations. Zinc (serum zinc < 9.9 μmol/L) and iron deficiency (serum ferritin < 20 mcg/L) were found in 76.4% and 10.9% of infants at 4 months, respectively. We found a positive association between zinc intake with infant weight parameters, and iron store at birth with serum ferritin of 4-month-old infants.

Conclusion: High proportion of infants had inadequate zinc intake and zinc deficiency. Iron intake among breastfed infants was adequate during the first 4 months. Iron status was influenced by iron store at birth, but not the breast milk iron intake during birth to 4 months.

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List of Abbreviations

AI	Adequate intake
ANC	Antenatal care
AR	Average intake
BMI	Body mass index
DRI	Dietary reference intake
EAR	Estimated average requirement
EFSA	European Food Safety Authority
FFQ	Food frequencies questionnaires
FLRF	Family Larsson Rosenquist Foundation
GA	Gestational age
GDM	Gestational diabetes melitus
Hb	Hemoglobin
Hct	Hematocrit
IAEA	International Atomic Energy Agency
IGF-1	Insulin like growth factor 1
IOM	Institute of Medicine
IQR	Interquartile range
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
WAZ	Weight-for-age Z-score

List of publications

Publication A

Dumrongwongsiri O, Winichagoon P, Chongviriyaphan N, Suthutvoravut U, Grote V, Koletzko B. Effect of Maternal Nutritional Status and Mode of Delivery on Zinc and Iron Stores at Birth. Nutrients. 2021;13(3):860. doi: 10.3390/nu13030860.

Publication B

Dumrongwongsiri O, Winichagoon P, Chongviriyaphan N, Suthutvoravut U, Grote V, Koletzko B. Zinc and iron adequacy and relative importance of zinc/iron storage and intakes among breastfed infants. Matern Child Nutr. 2021; e13268.

My contribution to the publications

1. Contribution to publication A

I provided all the document needed for ethical approval. I enrolled the participants at antenatal clinic and performed all processes of data collection. I handled the bio-samples before sending to laboratory or being kept frozen until analysis. I did the data management and performed initial statistical analysis. I discussed the results with supervisors and did the further statistical analysis to determine the final outcomes of the study. I drafted the manuscript and revised according to the supervisors' comments. After peer review process, I edited the manuscript and responded to the reviewers' comments.

2. Contribution to publication B

I performed all data collection processes, did the nutrient analysis from food data. I did the data management and statistical analysis. I discussed with supervisors to decide how to interpret and demonstrate our data in the manuscript. I drafted the manuscript and revised according to the supervisors' comments. After peer review process, I edited the manuscript and responded to the reviewers' comments.

1. Introductory summary

1.1 Background

1.1.1 Importance of zinc and iron in infants

Zinc is an essential nutrient in the human body involved in the function of many enzymes regulating major metabolic pathways[1]. Many studies reported an association of zinc deficiency and impaired linear growth of infants and young children[2]. A meta-analysis showed that zinc supplementation improved growth outcomes in infants and young children[3]. The association between zinc and growth can be explained by the fact that zinc is involved in DNA synthesis as well as cell proliferation and differentiation[4]. In addition, zinc deficiency can impair growth by reduction of insulin-like growth factor 1 (IGF-1) concentration[5]. Besides somatic growth, zinc also plays important roles in brain growth. It is involved in neuronal differentiation and migration, differentiation of oligodendrocytes and myelination, and release of neurotransmitters[6].

Iron is an important component of hemoglobin, with functions in transport of oxygen to body tissues and organs. Studies in animal models showed that iron is involved in brain development and iron deficiency can alter brain metabolism, myelination and neurotransmitter functions, especially during the brain growth spurt (during gestation and lactation period)[7]. Observational studies in infants aged under 2 years showed that iron-deficient infants had lower developmental test score in the cognitive, motor and social-emotional aspects[8].

1.1.2 Zinc and iron metabolism in infants during exclusive breastfeeding period

Infants are born with zinc and iron stores accumulated through trace element transfer via the placenta during intrauterine life, which represents source of nutrients for utilization during the early postnatal period of life.

Maternal zinc is transferred to fetus via the placenta. It was estimated that 57% of maternal zinc gain during pregnancy was transferred to fetus[9]. Placental zinc transport is tightly regulated, as it is found that cord blood had higher zinc concentration than maternal blood with the ratio of maternal/cord blood zinc concentration approximately 0.7[9]. Previous studies showed that preterm infants had lesser body zinc stores at birth which was due to decreased time for placental transfer[10-11]. There were some reports on an association of cord blood zinc and birth weight[12-13].

During pregnancy, iron is transferred via placenta to fetus, especially during the 3rd trimester. The placental transfer of iron is well regulated so that the iron stores of infants at birth are not dependant on maternal iron status or maternal anemia[14-15]. Infant iron stores at birth can be assessed by determination of cord blood ferritin[15].

Breast milk is the source of dietary zinc for infants during the exclusive breastfeeding period. An isotope study showed that fractional absorption of breast milk zinc in intestine of infants increased with age[16].

After birth, zinc and iron intakes of exclusively breastfed infants depend solely on breast milk. Together with daily intakes, infants also utilize zinc and iron from body stores to meet their requirements. Zinc storage may not have major contribution to zinc status of infants, as there were no reports of the association between zinc store at birth (cord blood zinc) and infant growth or zinc status after birth. In contrast to zinc, infants employ iron from storage together with dietary iron obtained from breast milk. A longitudinal study followed breastfed infants from 1 to 12 months of age showed that iron stores is the main source for iron utilization in infants together with little amount of dietary iron obtained from breast milk[17]. Breast milk contains low iron and provides minor supply for infant's iron need. Iron from breast milk, together with iron store and iron from recycled hemoglobin supposed to be adequate during the first 6 months of life[18].

1.3 zinc and iron deficiency in breastfed infants

Breast milk is the best nutrition for infants. Theoretically, it provides adequate nutrients for infants during the recommended exclusive breastfeeding period. However, many previous studies reported deficiency of zinc and iron among breastfed infants. A study of Thailand reported the prevalence of zinc deficiency in 4-6-month-old breastfed infants as 14.9%, while 5.3% of formula fed infant were zinc deficient[19]. Low zinc intake from breast milk may cause zinc deficiency in breastfed infants as the study found a correlation of breast milk zinc and infants' plasma zinc concentrations[19]. A previous report of zinc deficiency in breastfed infants showed that breast milk from mothers of zinc-deficient infants had zinc concentrations below the median value for lactation age[20]. A report, gathering data regarding iron status of breastfed infants from 6 studies, showed that the prevalence of iron deficiency among healthy 6-month-old breastfed infants was 19.7%, and the predictors of iron deficiency were male infants and lower birth weight[21].

1.2 Rationale and Objectives

1.2.1 Zinc and iron intakes from breast milk

Information of nutrient intakes among the healthy population is used for determination of dietary reference intakes (DRI). The recommended nutrient intake of infants under 6 months of age is usually based on the study of intakes from breast milk. The Institute of Medicine (IOM) estimated the nutrient intake among infants aged 0-6 months by average concentrations of nutrient in breast milk during 2-6 months postpartum reported in several studies, and assumption of an average breast milk intake of 780 mL/day[22]. The European Food Safety Authority (EFSA) used the data of breast milk nutrient composition and an average breast milk intake of 800 mL/day[23]. Currently, available information regarding zinc and iron intakes among 0-6 months old infants is not adequate to set the recommended dietary allowance (RDA), therefore, the intakes were recommended as adequate intake (AI). IOM and EFSA recommended AI for infants during their first 6 months as 2 and 0.27-0.3 mg, for zinc and iron respectively[23-24]. In Thailand, the data regarding zinc and iron intakes among breastfed infants are limited and not sufficient to set the recommended intakes in infants age under 6 months[25].

1.2.2 Assessment of zinc and iron intakes in breastfed infants

Many studies reported on breast milk zinc and iron levels[26-28]. Some studies reported an association of breast milk zinc concentration with zinc status in breastfed infants[19], but no association of breast milk iron concentration with infant's iron status. Breast milk nutrient concentration alone does not reflect infant's intake unless the volume of breast milk is quantified. The quantitative amount of zinc and iron intakes were rarely reported. To estimate the actual nutrient intakes among breastfed infants, assessment of breast milk nutrient levels together with the amount of breast milk intake are needed. Previous reports of nutrient intakes from breast milk estimated the volume of breast milk by various methods, including assumption from the value proposed by previous literature[29] ,using the consumption of breast milk intake by healthy infants based on their total energy expenditure[30], or test-weighing method[31]. The International Atomic Energy Agency (IAEA)[32] proposed the deuterium oxide dose-to-mother technique, an isotope method used for accurate assessment of breast milk volume among breastfeeding mother-infant dyads.

1.2.3 Factors contributing to zinc and iron status in infants

As mentioned in the introduction, the nutrient store at birth is a source of nutrients in addition to breast milk, for infants during their first 6 months of life. Nutrient stores may be related to maternal characteristics or nutritional status, as well as clinical characteristics during antenatal and perinatal period. Therefore, this study was designed as a longitudinal study. Therefore, the data collection began during the pregnancy period and continued during the lactation period.

In general, it is recommended to provide exclusive breastfeeding to 6 months. However, the infant feeding practice in Thailand usually gives some amount of complementary food after the age of 4 months. Therefore, we designed the study until infant age of 4 months instead of 6 months to determine the intake from breast milk and the association of breast milk nutrient intake and other outcomes.

It is recommended to provide exclusive breastfeeding to 6 months. However, some caretakers may introduce some foods or liquids other than breast milk to infants before 6 months of age. This common feeding practice makes the exclusive breastfeeding rate in Thailand as low as 14% (26.4% in Bangkok)[33]. Therefore, this study was designed to follow breast milk intake to 4 months to decrease contamination of intakes from other foods thereafter.

1.2.4 Objectives of this study

The study primarily aimed to quantify the amount of zinc and iron from breast milk among breastfed infants during exclusive breastfeeding period. We also aimed to evaluate the association between the zinc and iron intakes with the nutrient status and growth parameters of infants, the factors associated with breast milk zinc and iron concentrations, as well as the relationship of maternal zinc and iron status during pregnancy and the nutrient status of infants.

1.3 Methods

This was a longitudinal study, following women from the 3rd trimester of pregnancy to 4 months postpartum. Pregnant women, at gestational age (GA) of 28-34 weeks were enrolled at the antenatal care (ANC) clinic, Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand. Then, they and their infants were followed up at delivery, 2- and 4-month postpartum.

The sample size calculation showed that 120 participants are needed in this study. The study protocol was comprehensively described in a publication of study protocol (see Appendix).

In brief, data collection included demographic data, assessment of dietary intake and nutritional status of mothers and infants, determination of breast milk zinc and iron concentrations together with assessment of breast milk volume and nutrient status. Data collection was planned in each participant visit as shown in table 1.1.

Table 1.1 Data collection plan in each participant visit

Data collection	Pregnancy (28-34 weeks)	Delivery	Postpartum	
			2-month	4-month
Demographic and antenatal data	✓			
Perinatal data		✓		
Maternal dietary assessment	✓		✓	✓
Maternal anthropometric assessment			✓	✓
Infant anthropometric assessment			✓	✓
Breast milk samples for analysis of zinc and iron concentrations			✓	✓
Assessment of breast milk volume intake			✓	✓
Cord blood samples for analysis of zinc and ferritin levels		✓		
Maternal blood samples for analysis of zinc and ferritin levels	✓			✓
Infant blood samples for analysis of zinc, ferritin levels				✓

The volume of breast milk consumed by breastfed infant was assessed at 2 and 4 months postpartum. The most accurate method for determination of breast milk intakes is the use of stable isotope by deuterium oxide dose-to-mother technique, which was performed according to the protocol proposed by IAEA[32]. In the real study situation, there was a limitation of using a stable isotope technique for assessment of breast milk intakes. Some mothers decided to express their breast milk and feed their infants by bottle feeding. Under this condition, the stable isotope technique could not be performed accurately among mother-infant pairs with this feeding practice. Therefore, we decided to use the record of milk bottle weight before and after each feeding to evaluate the volume of breast milk intake.

Zinc and iron intakes from breast milk were estimated using the data of milk nutrient levels and quantitative amount of milk intakes. The intakes were calculated as daily and cumulative intakes. The latter may better reflect the association of intakes with other parameters such as growth and nutrient status. The calculation method of cumulative intakes was described in the publication B and shows in figure 1.1.

Zinc deficiency was defined as serum zinc concentration below 10.7 and 9.9 μmol/L for mothers and infants, respectively[34]. As serum ferritin is affected by inflammation and our study did not perform the analysis of inflammatory markers, we used 2 cut-off levels of ferritin to determine iron deficiency among 4-month-old breastfed infants as below 20 mcg/L as general suggestion[35] and below 30 mcg/L as suggested ferritin cut-off level with presence of infection[36].

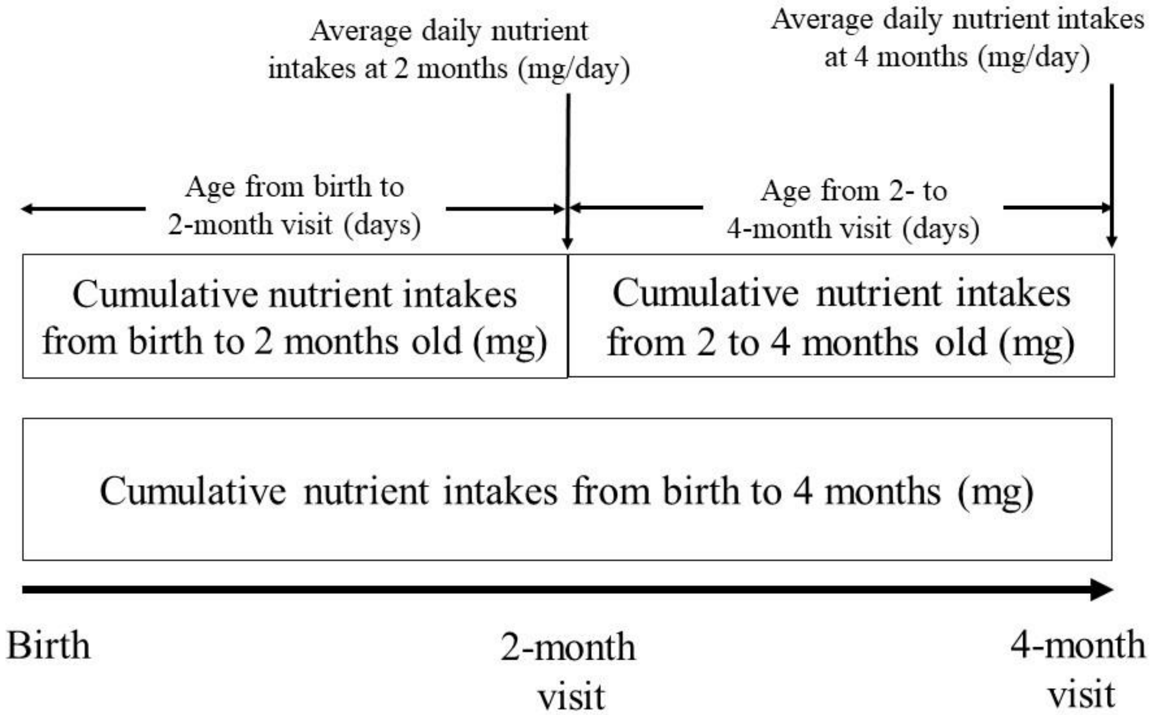

Figure 1.1 The method for determination of cumulative nutrient intakes

In general, the adequacy of nutrient intakes was determined by comparing with the estimated average requirement (EAR). Data from previous studies are not adequate to establish EAR of zinc and iron intakes among infant aged under 6 months. Instead, they are recommended as adequate intake (AI). Using AI to determine the adequacy of nutrient intakes may overestimate the prevalence of inadequacy. Therefore, it was proposed to calculate average requirement (AR) from AI by divided with 1.25 (AR=AI/1.25)[37].

1.4 Ethical consideration and conflict of interest

The study protocol was approved by the Human research ethic committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok (Protocol ID 03-60-31), and ethical committee, Ludwig Maximillians Universitaet, Munich (Project No. 18-015). All processes of the study complied with the declaration of Helsinki.

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1.5 Results

One hundred and twenty pregnant women were enrolled in the study during March 2018 to August 2019. Three pregnant women had emergency delivery at other hospitals and were excluded from the study. At 2 and 4 months postpartum, only 64 and 56 mothers provided full breastfeeding to their infants, respectively. The summary of number of participants and the reasons of exclusion from the study are shown in Figure 1.2. Most of participants were 20-35 years old. Approximately one-third of participants were elderly pregnancy (age over 35 years old). Most participants finished the bachelor's degree and had a family income of more than 30,000 baht/month. As the study site was in Bangkok, the capital city of Thailand, these characteristics may differ from the population in other parts of country. More than half of pregnant women carried their first child. The mean pre-pregnancy BMI was in normal range. However, one third of participant were obese (pre-pregnancy BMI $\geq$ 23 Kg/m^2)

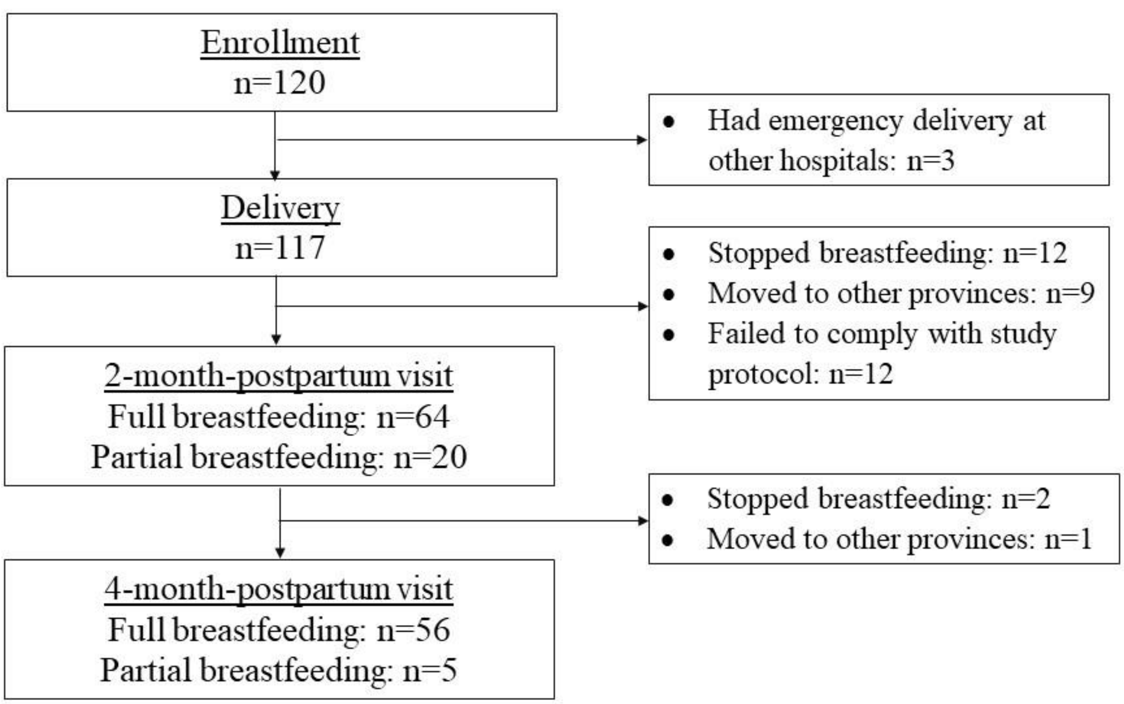

Figure 1.2 Participants in the study

This study was a longitudinal study during pregnancy and lactation. During pregnancy, the nutrients transfer from mother to infants via placenta and establish nutrient stores at birth which are utilized by infants during their early age. The first data analysis was done after the completion of data collection during pregnancy and delivery, which aimed to determine zinc and iron stores in infants at birth and the factors associated with the nutrient stores. The results of this analysis were summarized in publication A[38]. In brief, we described the nutrient stores at birth through the biomarker cord blood zinc and ferritin. We showed an association of maternal nutritional status presented as pre-pregnancy BMI with cord blood zinc and ferritin. Interestingly, vaginal delivery showed positive association with nutrient stores at birth.

The final analysis was performed after accomplishment of all data collection (until 4 months postpartum) and laboratory analysis. The results were reported in the publication B. We showed the breast milk zinc and iron levels at 2 and 4 months postpartum, and the quantitative amount of breast milk intakes. We presented the data as average daily zinc and iron intakes and compared with previously established recommended level to determine the adequacy of intakes. We also estimated the cumulative zinc and iron intake from birth till the end of study to determine the effect of intake on infant outcome such as growth parameters and biochemical status. The results showed that the percentage of infants who had zinc intakes below AR at 2 months old was 14.5% and increased to 40% at 4 months old. In contrast to zinc, the prevalence of 2-month-old infants with iron intakes below AR was only 1.6% and there was no infant with low iron intake at 4 months.

The associations between zinc and iron intakes from breast milk with infant growth parameters and biochemical status were determined as shown in publication B. Because nutrient stores at birth contribute to growth and nutrient status of infants during their early life, these factors were included to the regression analysis. We try to compare the magnitude of the association between nutrient intakes and storage with infant outcomes by using the relative importance. In summary, we found that zinc intakes showed stronger correlation with growth parameters than zinc store at birth and was positively associated with infant weight parameters (weight-for-age Z-score;WAZ and weight gain). In contrast, iron status of infants at 4 months old was positively associated with iron stores at birth, while no significant association with iron intake was found.

1.6 Discussion

This study was the first study assessing infant zinc and iron intakes from breast milk in Thailand. The results of this study show inadequacy of zinc intakes among a considerable number of breastfed infants during the exclusive breastfeeding period. Meanwhile, high prevalence of zinc deficiency among infants at 4 months of ages was reported. Iron intake was shown to be adequate compared to reference intakes, and a low proportion of infants had low serum ferritin levels.

This study adopted comprehensive techniques to quantify breast milk intakes. The deuterium oxide-dose-to-mother technique is widely used for assessing breast milk intakes and provides an accurate result. With the limitation due to breastfeeding practice, we try to obtain the accurate records of breast milk intake among infants fed with expressed breast milk via bottle feeding. Breast milk intakes reported in our study were similar with the result from previous study performed in Thailand[39]. Breast milk intake increased with the age of infants, but the relative breast milk intake per body weight of infant decreased from 2 to 4 months. Likewise, growth velocity of infants was significantly higher from 0-2 months compared with from 2-4 months. This implies that relative energy and nutrient requirement per body weight of infants is higher during the early period of life.

We analysed the association of nutrient intakes with biochemical zinc and iron status, and infant growth parameters. The analysis also included nutrient store at birth as a factor which also contributed to nutritional status and growth of infants during the first 6 months of life. We also use the relative importance to see how the magnitude of the association between each factor (nutrient stores or nutrient intakes) with the outcomes.

We found a positive association of cumulative zinc intake and weight parameters (WAZ, weight gain), but no association with serum zinc of infants. Zinc store at birth was not related with either infant growth parameters or serum zinc. Zinc intake is associated with growth in infants and children. Previous studies showed that zinc supplementation is positively associated with infant weight parameters among infant aged 6 months[40] and under 2 years old[3], while it had more effect of height gain among children aged over 2 years [3]. Assessment of zinc status by serum zinc concentration may have limitations, as it varies by recent meal, and may not reflect the whole-body zinc because of tightly homeostatic mechanism to enrich zinc in tissues and organs[34]. We did find zinc intake associated with serum zinc in breastfed infants.

In contrast to zinc, iron utilization in infants during the first 6 months seemed to depend more on iron stores at birth than iron intake during the first postnatal months. The study results that cumulative iron intakes had less relative importance to iron status and infant growth parameters. Breast milk iron concentration is low and may be not adequate for infants after 4-6 months of age. However, complementary food is an important source of dietary iron for infants beyond 6 months old. Our study determined iron status of infant at 4 months old and found that the prevalence iron deficiency was 10-30% depending on the ferritin cut off level applied, which is much lower than the prevalence of zinc deficiency. Cord blood ferritin was strongly associated with infant iron status at 4 months. Iron stores at birth may be adequate for infant's requirement until at least 4 months of age. Approaches to increase iron endowment in infants such as delayed cord clamping was shown to be positively associated with iron status during infancy[41-42]. A longitudinal study of iron status among breastfed infants showed that iron deficiency anemia was found in only 3.4% at the age of 4 months but was as high as 23.9% at the age of 6 months[43]. To prevent iron deficiency in breastfed infants, universal iron supplementation from the age of 4 months may not be necessary. Iron supplementation in the iron-sufficient infants may increase risk of iron overdose and toxicity, as previous studies showed that lack of iron homeostatic mechanism among infants aged under 6 months[18]. After 6 months of age, when the iron store is depleted, iron supplementation and iron-rich complementary food should be considered.

Nutrient intakes among infants during their early life are dynamic. Breast milk zinc and iron levels decreases during the lactation period. Infants consume more breast milk volume when they are growing up. However, this study showed that the breast milk zinc intake was decreasing from 2 to 4 months and the proportion of inadequate zinc intake was almost 3 times higher among infants at 4 months compared to 2 months of age. In contrast to zinc, breast milk iron rapidly declined during the first 14 days of lactation and then slightly declined from 14 days to 6 months[44]. This study measured breast milk iron at 2 and 4 months and found no difference between iron concentration in breast milk between these 2 time points. When breast milk iron intake was calculated, iron intake was not different between infants aged 2 and 4 months old. Iron intake among infants was adequate when compared with the AR.

As we found high prevalence of zinc deficiency and inadequacy of zinc intake among breastfed infants especially at 4 months, we tried to analyse factors associated with zinc concentration in breast milk. Similar to previous studies[26, 29, 45], our results show that zinc concentration in breast milk is independent of maternal age, dietary intakes, and nutritional status. However, we found that the decline in breast milk zinc concentration overtime was negatively associated with maternal body fat percentage. This means that lactating mothers with less body fat percentage had a larger decline in breast milk zinc concentration during the study period. With a larger decline in breast milk zinc, lesser amount of zinc would be transferred to breastfed infants at 4 months and might cause inadequacy of zinc intake. To our knowledge, no previous study determined the factors associated with the decline in breast milk zinc during the early period of lactation. It may be associated with maternal nutritional status or zinc intake, but our study may not have adequate power to determine this association. Further studies are needed to investigate the associated factors with the decline in breast milk zinc concentration.

Our study shows differences of zinc and iron metabolism during early life. Zinc stores at birth did not contribute much to zinc status of infants during their first 4 months. Daily zinc utilization may depend on dietary zinc intake, which we showed the association with infant growth parameters. To ensure good zinc status among breastfed infants, breast milk should have adequate zinc concentration, or alternatively, infant may be provided with supplementation. The decrease of breast milk zinc over the lactation period is interested. Further studies to identify the factors associated with this decline in breast milk zinc concentration may prevent inadequacy of zinc intake among breastfed infants during the first 4-6 months of age.

In contrast to zinc, iron status of infant was not associated with iron intake from breast milk. Iron store at birth had major contribution to iron status of infants during the first 4 months. Therefore, there were many recommendation to increase iron store at birth such as delayed cord clamping technique, in order to prevent iron deficiency among infants at the early age.

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2. Publications

2.1 Publication A

Dumrongwongsiri O, Winichagoon P, Chongviriyaphan N, Suthutvoravut U, Grote V, Koletzko B. Effect of Maternal Nutritional Status and Mode of Delivery on Zinc and Iron Stores at Birth. Nutrients. 2021 Mar 5;13(3):860. doi: 10.3390/nu13030860.

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Effect of Maternal Nutritional Status and Mode of Delivery on Zinc and Iron Stores at Birth

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Abstract: Zinc and iron deficiencies among infants aged under 6 months may be related with nutrient store at birth. This study aimed to investigate the association between zinc and iron stores at birth with maternal nutritional status and intakes during pregnancy. 117 pregnant women were enrolled at the end of second trimester and followed until delivery. Clinical data during pregnancy, including pre-pregnancy body mass index (BMI) and at parturition were collected from medical record. Zinc and iron intakes were estimated from a food frequency questionnaire. Serum zinc and ferritin were determined in maternal blood at enrollment and cord blood. Mean cord blood zinc and ferritin were 10.8 ± 2.6 µmol/L and 176 ± 75.6 µg/L, respectively. Cord blood zinc was associated with pre-pregnancy BMI (adj. ß 0.150; $p = 0.023$) and serum zinc (adj. ß 0.115; $p = 0.023$). Cord blood ferritin was associated with pre-pregnancy BMI (adj. ß −5.231; $p = 0.009$). Cord blood zinc and ferritin were significantly higher among those having vaginal delivery compared to cesarean delivery (adj. ß 1.376; $p = 0.007$ and 32.959; $p = 0.028$, respectively). Maternal nutritional status and mode of delivery were significantly associated with zinc and iron stores at birth. Nutrition during preconception and pregnancy should be ensured to build adequate stores of nutrients for infants.

Keywords: cord blood zinc; cord blood ferritin; iron deficiency; zinc deficiency; nutrition in pregnancy; pre-pregnancy BMI

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1. Introduction

Trace elements are essential for fetal growth and development. During late pregnancy, certain nutrients are transferred across the placenta to provide adequate nutrient stores for utilization during early infancy. Nutrient deficiencies during pregnancy were shown to contribute to pregnancy complications, adverse birth outcomes, and compromised nutrient storage in the newborn [1–3].

Zinc and iron are essential nutrients for fetal development. Zinc and iron content in fetal body were increasing with increased gestational age. Zinc and iron from maternal circulation are transferred through the placenta to provide these essential nutrients to the fetus, especially during the 3rd trimester. Maternal nutrient status during this period is crucial for the endowment of nutrient in the fetal body [1,3]. Cord blood zinc and iron levels are higher than maternal blood levels during pregnancy, reflecting active transport processes across the placenta [4,5]. Several studies showed a positive correlation of cord blood zinc with birth weight [6,7]. A systematic review and meta-analysis confirmed that both maternal zinc status during pregnancy and cord blood zinc were inversely associated with intrauterine growth restriction and low birth weight [8]. A study in preterm and term infants showed that cord blood zinc was positively correlated with gestational age [6]. As cord blood zinc level is associated with birth weight and gestational age, it may reflect the accumulation of zinc in fetus during prenatal life.

Cord blood ferritin reflects iron endowment of infants at birth and is associated with infant iron status thereafter [9,10]. Several studies reported a positive association of maternal iron status and cord blood ferritin level. Infants born to anemic or iron deficient mothers had lower cord blood ferritin compared to infants born to non-anemic/iron deficient mothers [11–13]. In addition, maternal anemia is also related to infant iron status after birth, but it is not clear whether this is due to low iron stores or poor postnatal diets. Exclusively breastfed infants born to anemic mothers not only had lower cord blood ferritin, but also lower serum ferritin at the age of 14 weeks [12].

During the first 6 months of life, infants receive zinc and iron from breastfeeding and utilize nutrient stores at birth to meet their needs. However, zinc and iron deficiencies were reported among infants aged less than 6 months [14,15], which may be related to insufficient nutrient stores at birth, low intakes of breast milk, or intakes of other foods with low contents of these micronutrients. In this study, we aimed to investigate whether cord blood zinc and ferritin levels were related to dietary intakes and status of zinc and iron status during pregnancy and other maternal and newborn factors at delivery.

2. Materials and Methods

This is a prospective observational study, following pregnant women from the third trimester until child delivery. Pregnant women who attended the antenatal care clinic (ANC) at Ramathibodi Hospital, Bangkok, Thailand, were enrolled at 28–34 weeks gestation. Inclusion criteria were singleton pregnancy, plan to deliver the baby at Ramathibodi Hospital, and intention to breastfeed. Exclusion criteria were emergency delivery elsewhere. The study protocol was explained to each participant. Once they understood the protocol and were willing to participate the study, written informed consent was obtained. The protocol was approved by human research ethic committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 03-60-31) and Ethical Committee, Ludwig Maximillian Universitaet, Munich (Project no. 18-015). All the processes of the study were performed according to the Helsinki Declaration.

This study is a part of the larger study following pregnant women from the third trimester to child birth, and infants until 4 months of age. More details of the study protocol were published elsewhere [16]. The sample size (n = 120) was determined in the original study, based on mean zinc intake in breastfed infants (1.00 $\pm$ 0.43 mg/day) reported by Krebs et al. [17]. Using the significant level of 0.05 and power of 0.8, the minimum sample size was 64. We estimated the possible drop-off of 45–50%. Therefore, the final sample size for recruitment was 120.

Clinical data during antenatal visits and at delivery were collected from medical records, including pre-pregnancy body mass index (BMI), weight gain during pregnancy, complications of pregnancy (i.e., pre-eclampsia, pregnancy induced hypertension and gestational diabetes (GDM)), supplementation during pregnancy, gestational age at delivery, mode of delivery (i.e., vaginal and cesarean delivery), and infant parameters. Other maternal characteristics, namely, family income and educational attainment were also collected using interview questionnaire. All pregnant women, regardless of age, were screened for GDM. Maternal smoking in Thailand is uncommon, and no participants reported smoking in this study.

Iron supplementation is routinely given to all pregnant women attending ANC, either iron tablet (ferrous fumarate; containing 66 mg of elemental iron), or multivitamin and mineral (Obimin-AZ; containing 20 mg of elemental zinc and 66 mg of elemental iron). The prescription was made by an obstetrician, based on the clinical profiles and drug tolerance by pregnant women. Average daily intakes of zinc and iron supplement were estimated by retrospective recalls of the dose and frequency of supplement ingested during the previous month. Dietary intakes were assessed at enrollment using a semiquantitative food frequency questionnaires (FFQ) by a skilled nutritionist. The FFQ is a comprehensive food list of commonly consumed foods by pregnant and lactating women in Bangkok, developed from a 3-day dietary record in a previous study done at Ramathibodi Hospital (Dumrongwongsiri et al., unpublished data). Nutrient intakes were calculated using INMUCAL software version 4.0 developed by the Institute of Nutrition, Mahidol University, based on nutrient contents in Thai foods, and expressed as iron or zinc intakes per day.

According to dietary recommended intakes (DRI) for Thais 2020 [18], the recommended zinc intake for pregnant women is 10.8 mg/day. For iron, there is no dietary recommended intake, but universal iron supplementation is recommended for all pregnant women throughout the pregnancy period.

Non-fasting peripheral blood samples were obtained at enrollment. Cord blood samples were obtained by obstetricians at the time of delivery from the placental side without milking. The practice of cord clamping depended on each obstetrician preference, and the data was not obtained in this study. The samples were collected from the cut surfaces of the cords, without placing a catheter into the vessels. Therefore, the samples represent mixed venous and arterial cord blood. Serum from both peripheral and cord blood samples was separated and kept at −80 °C until analysis. Serum zinc was analyzed by flame atomic absorption spectrophotometry (GBC Avanta S, GBC Scientific Equipment Pty Ltd., Dandenong, Australia). Serum ferritin was analyzed by a two-step chemiluminescent microparticle enzyme immunoassay on Architect i2000 SR (Abbott laboratories company, Lake Bluff, IL, USA). All containers and equipment used in blood samples collection were demineralized before used to avoid trace element contamination from environment. Zinc and iron deficiencies among pregnant women were defined as plasma zinc below 10.1 µmol/L [19] and serum ferritin below 15 µg/L, respectively [20]. Hemoglobin (Hb) concentration was taken from the ANC records at the first ANC visit, and at the beginning of the 3rd trimester of pregnancy. Hb below 11 g/dL was defined as anemia [21]. The cord blood ferritin values above 370 µg/L was excluded from the analysis due to the potential of inflammation [10].

Statistical analysis of the data was performed using SPSS version 18 (SPSS Inc. Released 2009 PASW Statistics for Windows, Version 18.0. SPSS Inc., Chicago, IL, USA). Descriptive data were presented as mean ± standard deviation (SD) and n (%). Comparison of means was performed by a Student t-test and one-way analysis of variance (ANOVA). Comparison of proportions was done by a chi-square test. Difference in Hb concentration and prevalence of anemia during 1st and 3rd trimester were analysed using a paired t-test and McNemar test. Correlation was determined by Pearson correlation coefficient. Factors which were correlated with cord blood zinc and ferritin, at p-value < 0.1 in univariate analysis, were selected for the multivariate analysis. Multivariate linear regression analysis was performed to determine factors associated with cord blood zinc and ferritin. Possible confounding factors including maternal age, infant sex, gestational age and birth order were included in the regression model. A backward elimination method with removal criteria at a p-value <0.10 was used for selecting the final regression models. Regression coefficient (ß) and 95% confident interval are presented. The p-value <0.05 was determined as statistically significance.

3. Results

One hundred and twenty pregnant women were enrolled at ANC. Three women had emergency deliveries at other hospitals and were excluded from the study. Hence, 117 pregnant women completed the study, and their characteristics and infant parameters are shown in Table 1. The rate of GDM and cesarean delivery in the study population were higher than in other areas of the country. All pregnant women were given iron supplementation, while 109 (93.2%) pregnant women were also given zinc supplements. Dietary zinc intake, without supplementation, was lower than Thai recommended daily allowances. Only 16.8% of pregnant women had adequate dietary zinc intake from the diet alone. When combined with prenatal supplementation, zinc intakes of 92.4% of pregnant women met the recommended daily allowance.

Table 1. Characteristics of study participants (*n* = 117)

Characteristics	Mean ± SD	*n* (%)
Before & During pregnancy		
Maternal age (years)	31.9 ± 5.6	
Pre-pregnant BMI (Kg/m^2)	22.0 ± 3.9	
<18.5		21 (17.9)
18.5–22.9		52 (44.5)
≥23		44 (37.6)
Zinc intake (mg): Diet Supplement Diet and supplement Iron intake (mg): Diet Supplement Diet and supplement	8.5 ± 3.0 17.2 ± 5.8 25.7 ± 6.8 11.2 ± 5.0 63.8 ± 19.1 74.9 ± 20.1	
Education level		
high school		20 (17.1)
bachelor degree		74 (63.2)
higher		23 (19.7)
Family income (baht/month)		
<10,000		5 (4.3)
10,000 – <30,000		39 (33.3)
>30,000		73 (62.4)
Birth order		
1st child		70 (59.8)
2nd child		41 (35.1)
3rd child		6 (5.1)
Complication of pregnancy		
gestational diabetes (GDM)		25 (21.4)
pregnancy induced hypertension/preeclampsia		1 (0.9)
Mode of delivery		
vaginal delivery		63 (53.8)
cesarean delivery		54 (46.2)
Birth parameters		
Gestational age at delivery (weeks)	38.5 ± 1.2	
Birth weight (g)	3095 ± 394	
Birth length (cm)	49.7 ± 2.1	
Low birth weight (<2500 g)		8 (6.8%)
Infant sex		
Male		63 (53.8)
Female		54 (46.2)

Maternal Hb, serum zinc and ferritin levels, and cord blood zinc and iron levels are shown in Table 2. During the 3rd trimester, the Hb concentration was significantly decreased when compared to Hb concentration at 1st trimester (11.7 ± 1.0 vs. 12.2 ± 1.0, respectively; $p < 0.001$). The prevalence of anemia increased in the 3rd trimester when compared to the 1st trimester (23.9 vs. 10.3 %, respectively; $p = 0.002$). More than half of participants had zinc deficiency (51.3%) while prevalence of iron deficiency (14.5%) was much lower.

Table 2. Maternal zinc and iron/anemia status during pregnancy and cord blood zinc and ferritin levels

Laboratory Parameters	Total N	Mean ± SD	*n* (%)
During 1st trimester of pregnancy:			
Hb, g/dL	116	12.2 ± 1.0	-
Prevalence of anemia, % [1]	116	-	12 (10.3%)
During 3rd trimester of pregnancy:			
Hb, g/dL	117	11.7 ± 1.0	-
Prevalence of anemia, % [1]	117	-	28 (23.9%)
Prevalence of iron deficiency, % [3]	117	-	17 (14.5%)
Prevalence of iron deficiency anemia, % [4]	117	-	6 (5.1%)
Serum zinc, μmol/L	117	11.1 ± 4.8	-
Prevalence of zinc deficiency, % [2]	117	-	60 (51.3%)
Serum ferritin, μg/L	117	32.3 ± 21.1	-
Cord blood			
Cord blood zinc, μmol/L	114	10.8 ± 2.6	-
Cord blood ferritin, μg/L [5]	105	176.7 ± 75.6	-

Hb: Hemoglobin; [1] Hb < 11.0 g/L, [2] serum zinc < 10.1 μmol/L, [3] serum ferritin < 15 μg/L, [4] Hb < 11.0 g/L and serum ferritin < 15 μg/L, [5] Total cord blood samples for ferritin = 110, excluded cord blood ferritin > 370 μg/L ($n = 5$).

Factors associated with cord blood zinc and ferritin levels (unadjusted and adjusted for potential confounders) are presented in Tables 3 and 4, respectively. Pre-pregnancy BMI was positively associated with cord blood zinc (adj ß 0.15, 95% confidence interval (CI) [0.02,0.28], $p = 0.023$) and negatively associated with cord blood ferritin (adj ß -5.23, 95% CI [−9.14, −1.33], $p = 0.009$). Pregnant women with vaginal delivery had significantly higher zinc and ferritin in cord blood, compared to those who had cesarean delivery (*p*-values 0.007 and 0.028, respectively). Maternal zinc status was significantly related to cord blood zinc (Table 3), but there was no association between maternal iron status (serum ferritin) or Hb and cord blood ferritin (Table 4).

Table 3. Factors associated with cord blood zinc

Factors	Unadjusted Model		Adjusted Model	
	ß (95%CI)	*p*-Value	ß (95%CI)	*p*-Value
Pre-pregnancy BMI	0.06 (−0.05, 0.19)	0.30	0.15 (0.02, 0.28)	0.023 *
Mode of delivery [1]	1.12 (0.18, 2.06)	0.020 *	1.38 (0.38, 2.38)	0.007 *
Maternal zinc status	0.10 (0.005, 0.20)	0.039 *	0.12 (0.02, 0.21)	0.023 *
Maternal age	−0.05 (−0.13, 0.04)	0.28	−0.05 (−0.14, 0.04)	0.30
Birth order [2]	−0.08 (−0.90, 0.72)	0.84	−0.05 (−0.91, 0.81)	0.90
Gestational age at birth	−0.05 (−0.44, 0.35)	0.80	−0.16 (−0.56, 0.23)	0.41
Infant sex [3]	−0.04 (−1.01, 0.92)	0.92	−0.32 (−1.28, 0.64)	0.51
Dietary zinc intake	−0.05 (−0.21, 0.11)	0.53	−0.09 (−0.25, 0.07)	0.28

* $p < 0.05$; [1] Mode of delivery; 0 = cesarean delivery, 1 = vaginal delivery; [2] Birth order; 1 = first child, 2 = second or more child; [3] Infant sex; 1 = male, 2 = female.

Table 4. Factors associated with cord blood ferritin

Factors	Unadjusted Model		Adjusted Model	
	ß (95%CI)	*p*-Value	ß (95%CI)	*p*-Value
Pre-pregnancy BMI	−5.62 (−9.32, −1.92)	0.003 *	−5.23 (−9.14, −1.33)	0.009 *
Mode of delivery [1]	50.31 (22.54, 78.07)	0.001 *	32.96 (3.68, 62.24)	0.028 *
Gestational age at birth	10.84 (−0.90, 22.58)	0.07	9.44 (−2.10, 20.98)	0.11
Maternal age	−1.75 (−4.44, 0.95)	0.20	0.53 (−2.24, 3.29)	0.71
Birth order [2]	−24.76 (−49.40, −0.12)	0.049 *	−9.23 (−35.07, 16.61)	0.48
Infant sex [3]	28.91 (−0.10, 57.91)	0.05	−0.32 (−1.28, 0.64)	0.51
Maternal serum ferritin	0.20 (−0.48, 0.87)	0.56	0.06 (−0.59, 0.71)	0.86
Maternal Hb during 1st trimester	−0.88 (−15.72, 13.96)	0.91	−3.27 (−17.63, 11.09)	0.65
Dietary iron intake	−0.38 (−3.33, 2.58)	0.80	−0.41 (−3.21, 2.40)	0.77

* $p < 0.05$; [1] Mode of delivery; 0 = cesarean delivery, 1 = vaginal delivery'; [2] Birth order; 1 = first child, 2 = second or more child; [3] Infant sex; 1 = male, 2 = female.

4. Discussion

In the population of pregnant women from Bangkok, Thailand that were studied, we found a high prevalence of zinc and iron deficiencies during the 3rd trimester. Intakes of zinc and iron from the diet alone during pregnancy in our study were inadequate to meet the needs, whereas an approximately adequate recommended intake of both nutrients was achieved in women who took prenatal supplementation. Although all women took iron supplements and more than 90% zinc supplements, a high proportion had inadequate levels of blood markers. This might be caused by the change in nutrient requirement and physiology during pregnancy. Recommended intake was established by the evidence of nutrient from dietary intake. The metabolism of zinc and iron in supplementation may differ from the natural nutrient. The appropriate doses of iron and zinc supplementation, to raise the levels of blood markers during pregnancy, needed further investigation. In addition, serum zinc may not be the best biomarker for determination of zinc status in the population.

The benefits of iron supplementation during pregnancy have been shown in both iron-depleted and iron-replete populations as pregnancy progresses [22]. On the contrary, the benefit of zinc supplementation during pregnancy has not been clearly demonstrated. It is estimated that very high proportion (82%) of pregnant women worldwide have inadequate zinc intake [23]. Zinc supplementation during pregnancy is not mandated by the Ministry of Public health in Thailand, but some prenatal supplements for pregnant women marketed in Thailand contain zinc (15–20 mg/d). Zinc intake and status among pregnant women in Thailand should be investigated further to determine whether a general supplementation policy would be advantageous.

Mean (±SD) cord blood zinc and ferritin among the study population were 10.8 ± 2.6 μmol/L and 176.7 ± 75.6 μg/L, respectively. Pre-pregnancy BMI, mode of delivery and maternal status in late pregnancy (28–34 weeks) were associated with cord blood zinc level. Pre-pregnancy BMI and mode of delivery were associated with cord blood ferritin level. We did not find any association of dietary intakes or supplementation with cord blood zinc and ferritin levels.

Our results show that pre-pregnancy BMI and maternal obesity (pre-pregnancy BMI > 23 Kg/m^2) were positively associated with cord blood zinc level. Previous studies on the relationship between maternal obesity and cord blood zinc showed mixed results. Al-Saleh et al. [24] reported higher cord blood zinc among pregnant women having higher pre-pregnancy BMI; whereas a case-control study of obese and lean pregnant women found no difference of cord blood zinc level between 2 groups [25]. We also found that maternal serum levels of zinc during late pregnancy were associated with cord blood zinc regardless of maternal pre-pregnancy BMI (Table 4). This finding is consistent with the result of a meta-analysis of 23 studies [8]. In addition, this meta-analysis showed that low cord blood zinc level was associated with increased risk of low birth weight. However, we did not find this association, possibly due to a small number of infants with low birth weight (data not shown). The reported associations underline the importance of an adequate zinc status during pregnancy which contributes to the fetal growth and fetal zinc storage. Mechanisms for the effects of zinc on fetal growth need to be further elucidated.

In contrast to zinc, our study showed that pre-pregnancy BMI, but not serum ferritin, was associated with cord blood ferritin level. Pre-pregnancy BMI and maternal obesity were negatively associated with cord blood ferritin and there was no association between maternal iron status or deficiency (based on serum ferritin concentration during the 3rd trimester) and cord blood ferritin in our sample. Several other studies showed significantly lower cord blood ferritin in obese compared to normal weight pregnant women [26–29]. Hepcidin, which is a regulator of iron homeostasis, was increased in obese pregnant women and associated with poor maternal iron status and impaired iron transfer to infants [30–32]. Previous studies found cord blood ferritin associated with iron status and inversely with anemia during pregnancy [9,12,33]. A very large study showed a small but significant correlation (r = 0.07 with $p < 0.001$, $n = 3247$) between maternal serum and cord blood ferritin [34], and stronger correlation was demonstrated among pregnant women with serum ferritin below the threshold level of the study (13.6 μg/L). Studies in animal models showed placental adaptation for iron transport in response to maternal iron deficiency or low iron intake [35,36].

Our study also found the mode of delivery associated with both cord blood zinc and ferritin levels. The results show that infants born by vaginal delivery had higher cord blood zinc and ferritin compared to those born bed by cesarean delivery (Tables 3 and 4). Similar to our results, previous reports found higher cord blood zinc among infants born by vaginal delivery when compared with elective cesarean delivery [37,38]. Cesarean delivery was associated with lower cord blood ferritin [39]. Other micronutrients such as copper, magnesium and manganese were also found to be higher in cord blood among pregnant women with vaginal delivery compared to elective cesarean delivery [37,38]. This might be explained by a lower placental transfusion during active labor among pregnant woman with cesarean section compared to vaginal delivery. This was demonstrated in a study showing a higher placental residual volume and lower Hb concentration in cord blood of a mother who has had a caesarian delivery [40].

There was no association between dietary intake and cord blood nutrient levels in our study. To the best of our knowledge, there is no published study showing a correlation between zinc intake during pregnancy and cord blood zinc. For iron, a randomized control trial of iron supplementation in pregnant women showed no difference in cord blood ferritin concentration between placebo and supplemental group [41]. An isotope study of iron transfer to the fetus during the 3rd trimester of pregnancy found a different iron transfer to the fetus between pregnant women with and without iron supplementation, but there was no difference between cord iron profiles [42]. Prenatal iron supplementation might be more critical to maintain maternal iron status and related functions, while there is less impact on iron transfer to the fetus. Therefore, to ensure an adequate iron status in early infancy, other measures may be needed such as delayed cord clamping, which was shown to effectively increase infant iron stores [43].

To the best of our knowledge, the present study is the first in Thailand investigated zinc and iron stores of infants at birth. The study results emphasized the significance of maternal nutrition which influences nutrition during early life. As we found pre-pregnancy BMI was related to zinc store of infants at birth, this demonstrated the importance of pre-conception nutrition status of women at reproductive age. The natural mode of delivery had some advantages on nutrient transfer during delivery.

There were some limitations in our study. Cord clamping methods, which are related to iron transfer during delivery, were not recorded in our study. Data regarding prenatal supplementation may be prone to some error because they were retrospectively recalled by participants, and the actual compliance was not monitored. Further studies should investigate the effect of maternal nutritional status and prenatal supplementation on micronutrient status of infants after birth.

5. Conclusions

Zinc and iron deficiencies were highly prevalent among pregnant women in this study. Dietary intakes during pregnancy were problematic. Zinc storage of infants was related with maternal zinc level during pregnancy. Pre-pregnancy BMI was associated with cord blood zinc and ferritin concentrations. Vaginal delivery may provide an advantage for micronutrient transfer during active labor. Maternal nutrition during preconception and pregnancy affects some of the nutrient stores in infants. Prenatal iron supplementation has been universally recommended where iron deficiency is highly prevalent to prevent serious consequences of maternal death associated with child birth. In addition to iron supplementation, zinc supplementation may be needed in light of the high prevalence of zinc deficiency during pregnancy, and its significant contribution to the zinc store as reflected by cord blood zinc. Further study is needed to assess the magnitude of zinc deficiency in the population and effective intervention to improve both maternal zinc status and the infant store.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. The study protocol was approved by Human Research Ethic Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 03-60-31) and Ethical Committee, Ludwig Maximillian Universitaet, Munich (Project no. 18-015).

Informed Consent Statement: The study protocol was informed to all participants. All questions from participants were answered and explained by researchers until clearly understood. The participants were informed that the results from the study would be published for scientific purposes, without any information which could lead to identification of each participant. All participants provided written informed consent before enrolment.

Data Availability Statement: Presented data are available on request from the corresponding author.

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2.2 Publication B

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ORIGINAL ARTICLE

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Zinc and iron adequacy and relative importance of zinc/iron storage and intakes among breastfed infants

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Abstract

Neonatal nutrient storage and supplies from breast milk contribute to nutrient status and growth of infants during their early life. This study investigated the adequacy of zinc and iron intakes among breastfed infants during the first 4 months and determined the relative importance of zinc/iron storage versus nutrient intakes with infant's biochemical status and growth. A longitudinal study followed lactating women and their breastfed infants from birth to 4 months postpartum. Cord zinc and ferritin concentrations, as indicators of nutrient storages, were determined. Zinc and iron intakes from breast milk were determined by measurement of breast milk volume together with milk zinc and iron concentrations at 2 and 4 months postpartum. Inadequacy of nutrient intakes was determined using average requirement (AR) which were 1.6 and 0.24 mg/day for zinc and iron respectively. Infant's serum zinc and ferritin were determined at 4 months of age. The data were collected from 64 and 56 participants at 2 months and 4 months postpartum. Inadequate zinc intake was found in 14.5 and 40% of infants at 2 and 4 months old, respectively. The prevalence of biochemical zinc and iron deficiency in infants were 76 and 11%, respectively. Iron endowment was significantly associated with serum ferritin at 4 months. The cumulative zinc intake was positively associated with weight gain and weight-for-length Z-score, but not length. This study provides quantitative data on zinc and iron intakes, and demonstrates the relative importance of nutrient storage versus intakes on biochemical status and growth of breastfed infants.

KEYWORDS
breast milk iron concentration, breast milk zinc concentration, cord blood ferritin, cord blood zinc, iron intake, zinc intake

1 | BACKGROUND

Several micronutrients, while required in minute amounts, have essential functional importance to bodily functions. Among them, two micronutrients of public health concerns are zinc and iron, which both are essential for infant growth and development. Stunting and iron deficiency anaemia among infants and young children have been global public health problems and still common in low and middle-income countries (Lutter, 2008; Tzioumis et al., 2016). Zinc has structural roles for proteins and peptides and is a component of numerous enzymes with relevance for metabolism and immunity (Prasad, 2013). Zinc deficiency in infants results in poor growth and brain development (Brion et al., 2020; Mattei & Pietrobelli, 2019; Prasad, 2013). Similarly, iron is essential for haemoglobin, myoglobin and neurotransmitter synthesis and plays an important role for brain development (Mattei & Pietrobelli, 2019). Iron deficiency during early life can lead to irreversible functional alterations of the developing brain (Lozoff & Georgieff, 2006). During gestation, especially in the third trimester of pregnancy, materno-fetal transfers of these trace elements provides a zinc and iron endowment in the neonate covering much of needs during the early postnatal period (Brion et al., 2020; Siddappa et al., 2007). After birth, breast milk is the sole supply of these nutrients if the infants are fully breastfed during the first 6 months. It has been postulated that neonatal zinc and iron body store, together with zinc and iron providing from breast milk are adequate for covering the needs of term breastfed infants during the first 6 months after birth. (Fewtrell et al., 2017; Krebs et al., 2014).

Neonatal zinc and iron body stores depended on several factors, such as maternal nutrition during pregnancy, mode of delivery and gestational age at delivery (Dumrongwongsiri et al., 2021; El-Farrash et al., 2012). Cord blood concentrations of zinc and iron reflect the pool size of intrauterine accretion (Siddappa et al., 2007; Terrin et al., 2015) Previous studies showed that cord blood zinc concentration was positively associated with intrauterine growth, that is, higher birth weight, as well as pregnancy outcomes (Akdas & Yazihan, 2020; Gómez et al., 2015). Iron store was shown to be related with iron status later in infancy. A study in China showed that cord blood iron profiles was positively associated with iron status of infants at 9 months of age (Shao et al., 2021).

Exclusive breastfeeding is recommended during the first 6 months of life. Exclusively breastfed infants receive their zinc and iron supply solely from breast milk. Zinc and iron intakes can be assessed by measuring milk concentration of these nutrients along with the volume of breast milk intake. The Institute of Medicine (IOM) (Trumbo et al., 2001) and the European Food Safety Authority (EFSA) (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2013) estimated the adequate intake (AI) for zinc and iron for infants up to the age of 6 months to be 2 and 0.27–0.3 mg/day, respectively. These AIs were derived from breast milk zinc and iron concentration and estimated breast milk intake (780 ml/day as per WHO) obtained mainly in European infants and used for establishing recommended daily intakes by World Health Organization (UNICEF, 1998). However, data from other parts of the world are scarce.

Zinc and iron concentrations in breast milk were not associated with maternal nutrient intakes, nutrient status and supplementation (Aumeistere et al., 2018; Dror & Allen, 2018). Previous studies showed a rapid decline of zinc and iron concentrations in breast milk during the first 6 months of lactation (Han et al., 2011; Mello-Neto et al., 2010), whereas breast milk intake tends to increase during this period. Only a few studies reported total zinc and iron intakes among infants during the first 6 months by actually assessing both breast milk volume and nutrient concentrations (Daniels et al., 2019; Samuel et al., 2014). Assessing breast milk intake during exclusive breastfeeding is challenging as mothers may feed their babies directly from breast or feed expressed breast milk using a cup or bottle. Test-weighing methods are used to determine the amount of breast milk consumed by infants, by weighing infants before and after each feed and summing all feeds to obtain the total intake per day. It was also recommended that the measurements should be done continuously for 48–72 hours (Borschel et al., 1986). Hence, this method is not easily applicable as it markedly disturbs mother's routines regarding infant care and chores at home. As an alternative, the deuterium oxide dose-to-mother was developed and promoted by the International Atomic Energy Agency (IAEA) to assess breast milk intakes among breastfed infants (International Atomic Energy Agency (IAEA), 2010).

Key messages

- High prevalence of inadequate zinc intake was found among healthy breastfed infants at 4 months of age. A reduction in milk zinc concentration along the lactation period caused the decline in zinc intake even though infants consumed a higher breast milk volume.
- Cumulative zinc intake from birth to 4 months was associated with infant weight gain and weight parameters at 4 months of age, but not length.
- Neonatal iron storage had a stronger effect on serum ferritin of infants at 4 months old than to iron intake from breast milk, birth weight and infant gender.
- Understanding the contribution zinc/iron storage at birth and intake from breast milk to infant nutrient status provides clearer picture of zinc and iron metabolism in infant's body and has advantage in promoting good zinc and iron status among breastfed infants.

Although fetal zinc and iron accretion along with supplies from breast milk have been assumed to cover the infant needs during the early life. A measure of how importance of zinc and iron storage at birth and postnatal intakes has not been examined empirically. In this study, we used a relative importance as a mean to compare how much of our interested factors (i.e., nutrient storages and breast milk supplies) contributed to infant nutrient status. Therefore, we aimed to quantitatively determine the adequacy of zinc and iron intakes among breastfed infants during the exclusive breastfeeding period by measuring breast milk zinc and iron concentrations and breast milk intakes of infants during the first 4 months. In addition, we wished to determine the relative importance of zinc/iron stores at birth reflected by cord blood concentrations versus zinc and iron intakes from breast milk with biochemical status and growth among breastfed infants at 4 months of age.

2 | METHODS

We performed a prospective observational study from pregnancy to 4 months postpartum. The study enrolled pregnant women at 28–32 weeks of gestation at the antenatal care clinic, Ramathibodi Hospital, Bangkok, Thailand. Women were followed at delivery, and at 2 and 4 months postpartum. The inclusion criteria was healthy pregnant women planned to deliver their babies at Ramathibodi Hospital, lived in Bangkok Metropolitan area. Pregnant women carried twin or trippet and who had contraindication of breastfeeding were excluded. The study protocol was previously published (Dumrongwongsiri et al., 2020). According to the estimation of sample size published in the study protocol, this study needed 64 participants to compete the study. We estimated 50% dropped out rate and then we totally enrolled 120 pregnant women in our study. Here we report on the evaluation of data after delivery and postpartum visits, including only breastfed infants who had not receive any formula or other foods.

In brief, demographic data and perinatal data including mode of delivery, birth weight and length, and perinatal complications were obtained from medical records. Cord blood samples were collected during delivery for determination of zinc and ferritin concentrations. At 2 and 4 months postpartum visits, the data collection included maternal dietary intakes (by food frequencies questionnaires; FFQ), anthropometric measurement of mothers and infants, collection of breast milk samples, and assessment of infant breast milk intakes. At 4 months postpartum, infant blood samples were collected for serum zinc and ferritin analysis.

2.1 | Breast milk samples collection and determination of breast milk zinc and iron concentrations

Breast milk samples were collected at 2 and 4 months postpartum. Lactating women were asked to empty one breast by using a hospital-grade, electric breast pump (Medela Lactina, Medela, Bangkok, Thailand). After emptying the breast, the milk collection was thoroughly mixed and 10–15 ml of breast milk were transferred to plastic tubes and kept at −80°C until analysis. All equipment and containers used for breast milk collection were treated with nitric acid solution to prevent trace element contamination before use. Breast milk was digested with HNO_3. Then, breast milk zinc (BMZn) and iron (BMFe) concentrations were determined by using inductively coupled plasma-mass spectrometry (ICP-MS) (McKinstry et al., 1999)

2.2 | Determination of volume of breast milk intake by infants

Infant breast milk intake was determined at 2 and 4 months postpartum. The methods used to assess breast milk volume depended on feeding practice of each mother-infant pair. For lactating women who fed their infants directly from breast, the deuterium oxide dose-to-mother technique was applied. The study strictly followed the protocol of the IAEA (Dumrongwongsiri et al., 2020; IAEA, 2010). The stable isotope technique could not be applied in lactating women who expressed their milk and fed their infants via bottles. In these women and infants, a prospective 24-hour record of breast milk intake through three consecutive days. Researchers provided a precise kitchen digital scale (Tanita kitchen scale, Central Trading Company, Bangkok, Thailand) to every participant. At each feed, mothers recorded the weight of milk bottles before and after each feeding (milk bottles with or without milk leftover). Since breast milk intake was measured as weight (g), we converted to the milk intake to volume by using the specific gravity of breast milk (1.031) (Lawrence & Lawrence, 2011).

2.3 | Estimation of zinc and iron intakes from breast milk

Daily zinc and iron intakes from breast milk at 2 and 4 months postpartum were calculated by using the data of BMZn and BMFe multiplied by volume of breast milk intakes for each participant at each visit. The adequacy of zinc and iron intakes among infants was assessed based on reference values. Since the recommended nutrient intake of zinc and iron in infants aged up to 6 months are based on the AIs, which are greater than estimated average requirements (EAR), the used of AI may over estimate prevalence of inadequacy in study population (Institute of Medicine, 2000). Calculating average requirements (AR) from AI (calculated $AR = AI/1.25$) was proposed for assessing the adequacy of nutrient intakes in a population when EAR is not available (Allen et al., 2020). In this study, we used the calculated AR for zinc (1.6 mg/day) and iron (0.24 mg/day), to determine the adequacy of intakes in breastfed infants.

Cumulative zinc and iron intakes from birth to 4 months of age were estimated and used for assessing the association of nutrient intakes with nutrient status and growth. Nutrient intakes from birth to 2 months old were calculated from the average daily nutrient intakes measured at 2 months multiplied by infant's age in days at the 2-month visit. Nutrient intakes from 2 to 4 months old were calculated from average daily nutrient intakes at 4 months multiplied by age in days from the 2- to 4-month visits. The cumulative nutrient intakes from birth to 4 months of age were the summation of the calculated nutrient intakes during these two periods.

2.4 | Biochemical analysis

Zinc and iron endowment was measured by cord serum zinc and ferritin concentrations at delivery. Infant's zinc and iron status was measured by serum zinc and ferritin concentrations at 4 months of age. Serum zinc concentration was analysed by a flame atomic absorption spectrophotometry (GBC Avanta S, GBC Scientific Equipment Pty Ltd., Dandenong Australia) (Smith et al., 1979). Serum ferritin concentration was analysed by chemiluminescent immunoassay. Zinc deficiency was defined as serum zinc below 9.9 μmol/L (King et al., 2015). Because there is a suggested cut-off level of serum ferritin with and without presence of infection, iron deficiency was determined using the cut-off level of serum ferritin as below 30 mcg/dL (Lynch et al., 2018) and below 20 mcg/L (Mattiello et al., 2020). Inflammatory markers were not determined in this study. Instead, we screened the history of previous illness during 2 weeks before every data collection visits in all participants.

2.5 | Anthropometric assessment

Anthropometric measurements were performed in both lactating women and their infants. Weight and height measurements of lactating women were performed. Body composition of lactating women was assessed by bioelectrical impedance analysis (InBody 720; INBody, Cerritos, CA, USA). Infant weight was measured to the nearest 10 g by infant digital scale. Infant length was measured by a wooden board with sliding foot piece to the nearest 0.1 cm. Growth was measured as the total weight and length gain from 0 to 4 months of age were calculated by subtraction of birth weight and length from respective measurements at 4 months. Infants' growth parameters at 4 months of age were calculated to Z-scores (weight-for-age Z-score; WAZ, length-for-age Z-score; LAZ, weight-for-length Z-score; WLZ) based on WHO growth standard, and performed by WHO Anthro calculator (https://www.who.int/tools/child-growth-standards/software).

2.6 | Data analysis

Kolmogorov–Smirnov tests were used to assess normality distribution of all variables. The descriptive data are presented as percentage, mean (±SD) or median (interquartile range; IQR). The cumulative zinc and iron intakes are presented as mean (±SD) and median (IQR). The volumes of breast milk intakes at 2 and 4 months were compared by paired t-test. Both BMZn and BMFe intakes were not normally distributed, hence paired t-test of log-transformed variables was used to compare these values between 2 and 4 months of age. Bivariate analysis was used to assess the associations between maternal factors (including age, anthropometric measurement and dietary intakes) and breast milk nutrient concentrations at each time point, or the differences between the two time points.

Multivariate linear regression analysis was performed to determine the association between zinc/iron storage at birth (cord concentrations) and cumulative intakes and biochemical status or growth at 4 months. Standardized beta coefficients were used to judge the relative importance between the storage and cumulative nutrient intakes on biochemical status or growth. All independent variables were transformed to SD score before entering the regression model. We analysed the difference of R^2 of the regression model when each interested factor was entered, to demonstrate how much the contribution of the factor to the model. The base model was done using the potential confounding factors to the dependent variable were entered. Then, each of the interesting variable was entered to the model. The value of R^2 of each model was record. The difference of R^2 (delta R^2) was calculated by subtraction of R^2 of the base model from the variable-entered model. Greater change of R^2 implied that the factor had more contribution to the model. All analysis were controlled for potential confounding factors. *P* value less than 0.05 was considered statistical significance.

2.7 | Ethical considerations

The protocol was approved by Human Research Ethic Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 03–60-31) and Ethical Committee, Ludwig Maximillian Universitaet, Munich (Project no. 18–015). All the processes of the study were performed according to the Helsinki Declaration.

3 | RESULTS

Among the 117 participants enrolled into the study, 64 and 56 mother-infants pairs completed the study visits at 2 and 4 months postpartum, respectively (Figure 1). Table 1 shows the demographic and perinatal data of study participants. The participants came from various socio-economic status. The participants excluded from the study had similar demographic and perinatal data as the participants who completed the study (data not shown). At 2 and 4 months of lactation, there were 42 (65.6%) and 25 (44.6%) of lactating women who continued using prenatal micronutrient supplements as Obimin AZ® (Zuellig Pharma, Singapore: provides 15 mg zinc and 66 mg iron per daily dose). The stable isotope technique was used for assessing breast milk intake in 39 of 64 (60.9%), and 17 of 56 (30.4%) participants at 2 and 4 months, respectively. The breast milk volumes determined by the stable isotope technique or by recording weighed milk bottles were not significantly different. (698 ± 185 vs 654 ± 115 ml/day; $p = 0.190$ for stable isotope technique and recording weighed milk bottles, respectively at 2 months, and 828 ± 187 vs. 757 ± 168 ml/day; $p = 0.179$ for stable isotope technique and recording weighed milk bottles, respectively at 4 months).

Regarding child growth and nutritional status, most of infants had growth parameters in the reference range. Weight and length gain during 0–2 months tended to be higher than those during 2–4 months (not significantly different). Prevalence of underweight, stunting, and wasting and were 4.7, 1.6 and 3.1%, respectively at 2 months and 5.4, 0, and 7.1%, respectively at 4 months (Table 1).

Bivariate analysis showed that BMZn and BMFe were not correlated with maternal age, body composition and maternal dietary intakes. BMZn and BMFe were similar among participants with different socio-economic status (data not shown). BMZn at 4 months was significantly lower than at 2 months, with a mean difference of 1.79 mg/L (95% CI [1.22, 2.35], $p < 0.001$) (Table 2). The difference of BMZn between 2 and 4 months was negatively associated with

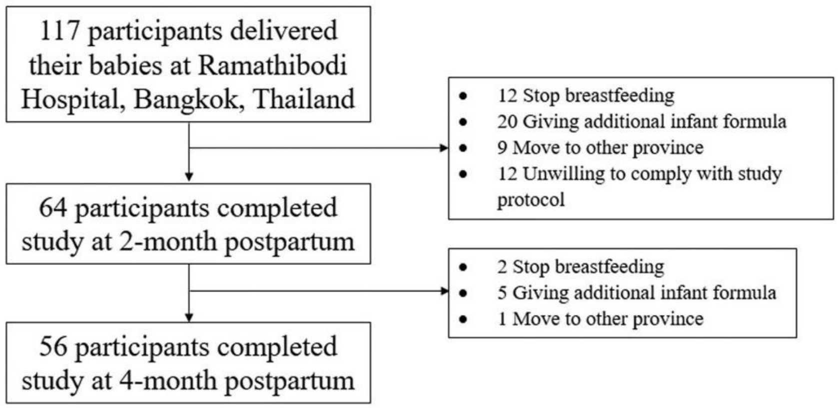

FIGURE 1 Participants in the study

TABLE 1 Demographic, perinatal characteristics and growth and nutritional status of study participants

Variables	Participants completed study at 2 months postpartum ($n = 64$)	Participants completed study at 4 months postpartum ($n = 56$)
Maternal age (y)	32.8 ± 5.1	32.6 ± 5.0
Pre-pregnancy BMI (kg/m^2)	21.9 ± 3.8	21.9 ± 3.4
Family income (Thai baht/month)		
Below 10,000	3 (4.7%)	2 (3.6%)
10,000–30,000	17 (26.6%)	14 (25%)
Over 30,000	44 (68.8%)	40 (71.4%)
Birth order		
1st child	36 (56.3%)	31 (55.4%)
2nd child	25 (39.1%)	22 (39.3%)
3rd or more child	3 (4.7%)	3 (5.4%)
Gestational age (week)	38.4 ± 1.1	38.3 ± 1.1
Infant gender—male	35 (54.7%)	33 (58.9%)
Birth weight (g)	3,138 ± 399	3,158 ± 392
Birth length (cm)	50.0 ± 1.9	50.1 ± 1.8
Low birth weight (<2,500 g)	4 (6.3%)	5 (8.9%)
Infant growth parameters		
Total weight gain from 0 to 4 months (g)		3,457 ± 697
Average weight gain from 0 to 2 months (g/day)	32.48 ± 7.42	
Average weight gain from 2 to 4 months (g/day)		21.54 ± 6.56
Total length gain from 0 to 4 months (cm)		13.34 ± 2.07
Average length gain from 0 to 2 months (cm/week)	0.85 ± 0.22	
Average length gain from 2 to 4 months (cm/week)		0.61 ± 0.15
Weight-for-age Z-score (WAZ)	−0.36 ± 0.89	−0.36 ± 0.88
Length t-for-age Z-score (LAZ)	−0.17 ± 0.88	−0.07 ± 0.73
Weight-for-height Z-score (WLZ)	−0.24 ± 0.97	−0.39 ± 1.06
Prevalence of underweight (WAZ < −2)	3 (4.7%)	3 (5.4%)
Prevalence of stunting (LAZ < −2)	1 (1.6%)	0
Prevalence of wasting (WLZ < −2)	2 (3.1%)	4 (7.1%)

TABLE 2 Zinc and iron concentration in breast milk, breast milk intakes, cord blood concentrations and infant's zinc and iron status

	2 months (n = 64)		4 months (n = 56)		
Breast milk	**Mean ± SD**	**Median (IQR)**	**Mean ± SD**	**Median (IQR)**	***p* value**
Zinc concentration (mg/L)	4.85 ± 2.37	4.30 (3.33–5.77)	2.99 ± 1.34	2.67 (1.86–3.80)	0.008[a]
Iron concentration (mg/L)	1.69 ± 1.11	1.27 (1.10–1.91)	1.48 ± 1.12	1.07 (0.72–1.89)	0.016[a]
BM volume (ml)	671 ± 166	669 (565–780)	782 ± 177	750 (662–884)	<0.001
BM intake per kg bodyweight (ml/kg)	129 ± 33	125 (107–140)	118 ± 25	117 (99–135)	0.001
Nutrient intakes	2 months (n = 62)		4 months (n = 50)		
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Zinc intake[b] (mg/day)	3.21 ± 1.72	2.88 (1.82–4.17)	2.26 ± 1.15	1.77 (1.38–2.92)	<0.001[a]
Zinc intake below AR[c] *n* (%)	9 (14.5%)		20 (40.0%)		
Zinc intake per kg bodyweight (mg/kg)	0.62 ± 0.32	0.54 (0.36–0.77)	0.34 ± 0.16	0.29 (0.20–0.48)	<0.001[a]
Cumulative zinc intake (0–4 m)[d] (mg)			361 ± 157	307 (254–417)	NA
Iron intake[b] (mg/day)	1.18 ± 0.89	0.91 (0.57–1.34)	1.14 ± 1.08	0.79 (0.53–1.34)	0.296[a]
Iron intake below AR[c] *n* (%)	1 (1.6%)		0		
Iron intake per kg bodyweight (mg/kg)	0.23 ± 0.18	0.16 (0.11–0.26)	0.17 ± 0.17	0.12 (0.08–0.19)	0.003[a]
Cumulative iron intake (0–4 m)[d] (mg)			152 ± 109	125 (91–165)	NA
Cord blood concentration			At birth (n = 56)		
Cord blood zinc concentration (μmol/L)			10.60 ± 2.62		
Cord blood ferritin concentration (μg/L)			181.59 ± 78.32		
Infant's biochemical status			4 months (n = 55)		
Serum zinc (μmol/L)			8.6 ± 2.1		
Prevalence of zinc deficiency n(%)[e]			42 (76.4%)		
Serum ferritin (μg/L)			74.4 ± 56.3		
Prevalence of iron deficiency *n*(%)					
Serum ferritin < 20 mcg/L[f]			6 (10.9%)		
Serum ferritin < 30 mcg/L[g]			16 (29.1%)		

[a]Paired *t* test of log-transformed variable.
[b]Zinc/iron intake, mg/d was calculated by BM volume × BMZn or BMFe concentration.
[c]AR is calculated average requirement derived from AI/1.25 (Allen et al., 2020).
[d]Cumulative intake (0–4 m) = [Zinc or iron intake/d at 2 m x #days (0–2 m)] + [Zinc or iron intake/d at 4 m × #days (2–4 m)].
[e]Serum zinc below 9.9 μmol/L (King et al., 2015).
[f]Serum ferritin below 20 mcg/L (Mattiello et al., 2020).
[g]Serum ferritin below 30 mcg/L (Lynch et al., 2018).

maternal percentage of fat mass ($\beta = -0.07$ [-0.146, -0.003], $p = 0.041$), that is, mothers with larger fat stores showed a lesser decline of milk zinc concentration. A stronger association was found after adjusting for maternal age and dietary zinc intakes ($\beta = -0.10$ [-0.17, -0.03], $p = 0.009$).

Breast milk volume and daily zinc and iron intakes among breastfed infants are shown in Table 2. Breast milk intake was significantly higher in male compared to female infants, both at 2 months (720 ± 150 vs. 612 ± 168 ml/day; $p = 0.010$) and 4 months postpartum (846 ± 177 vs. 686 ± 131 ml/day; $p = 0.001$). However, the breast milk intake per kg body weight at both 2 months and 4 months were not significantly different between genders.

The average daily zinc and iron intakes per kg body weight decreased significantly from 2 to 4 months postpartum. When using calculated AR (AI/1.25) to determine the prevalence of inadequate nutrient intakes in breastfed infants, 14.5% and 40% of the study infants had inadequate zinc intake at the age of 2 and 4 months, respectively, and the prevalence of zinc deficiency at 4 months of age was very high (76.4%). In contrast, daily iron intake was adequate at both ages in this study population, but the prevalence of iron deficiency is 10.9% (serum ferritin below 20 mcg/L) or 29.1% (serum ferritin below 30 mcg/L).

The relative importance of cord zinc/iron concentration and cumulative zinc/iron intakes on biochemical zinc/iron status and growth at 4 months are shown in Tables 3–5. There was no significant association between zinc storage at birth or cumulative intakes and serum zinc, although the effect size (standardized beta coefficient) of cord zinc concentration was larger than that of the cumulative intakes, controlling for confounding factors (Table 3). On the contrary, iron storage at birth was significantly associated with serum ferritin at 4 months, whereas the cumulative intake of iron was not. Higher birth weight and female gender were also significantly associated with higher serum ferritin. However, the analysis demonstrated a stronger effect of iron storage on serum ferritin than birth weight and gender (shown by a larger standardized beta coefficient) (Table 3). Analysis of R^2 of the regression model also showed greater difference when enter the variable cord blood ferritin (delta $R^2 = 0.358$) compared with cumulative iron intake (delta $R^2 = 0.003$) (Table S1).

Considering growth outcomes, the cumulative zinc intake was positively associated with total weight gain and WLZ, but not total length gain and LAZ (Table 4). Delta R^2 when entering the variable cumulative zinc intake were greater than cord blood zinc in both the model for total weight gain and WLZ (Table S1). In contrast, cord blood zinc concentration was not associated with any of the growth. Neither iron storage nor cumulative intake was associated with any anthropometric status (Table 5).

4 | DISCUSSION

This study reports quantitative zinc and iron intakes among breastfed infants in Thailand during the first 4 months of life. We demonstrated a positive association of cumulative zinc intake from breast milk with

TABLE 3 Associations between cord blood zinc and cumulative zinc intake (0–4 months) with infant's serum zinc at 4 months

	Serum zinc at 4 m				Serum ferritin at 4 m		
Variables (SD score)	β [95% CI]	*p* value	Std. Beta[b]	Variables (SD score)	β [95% CI]	*p* value	Std. Beta[b]
Cord blood zinc	0.46 [−0.16, 1.09]	0.142	0.222	Cord blood ferritin	37.64 [24.79, 50.49]	<0.001*	0.661
Cumulative zinc intake (0–4 m) (mg)	0.27 [−0.40, 0.94]	0.419	0.116	Cumulative iron intake (0–4 m) (mg)	3.70 [−8.55, 15.96]	0.545	0.067
Age at the end of study (days)	0.59 [−0.03, 1.21]	0.061	0.281	Age at the end of study (days)	−3.17 [−18.08, 11.73]	0.670	−0.046
Birth weight (g)	0.51 [−0.12, 1.14]	0.107	0.248	Birth weight (g)	16.02 [3.74, 28.31]	0.012*	0.282
Infant gender[a]	0.91 [−0.32, 2.13]	0.142	0.212	Infant gender[a]	26.71 [1.38, 52.04]	0.039*	0.227

Note. Independent variables were transformed to SD score before entering the regression model.
[a]Infant gender: male = 1; female = 2.
[b]Standardized beta coefficients.
*$p < 0.05$.

TABLE 4 Associations between cord blood zinc and cumulative zinc intake (0–4 months) with infant's growth and nutritional status at 4 months

Variables (SD score)	Growth parameters[a]							
	Total weight gain (g)[b]		Total length gain (cm)[b]		LAZ		WLZ	
	β [95% CI]	Std. Beta[c]	β [95% CI]	Std. Beta[c]	β [95% CI]	Std. Beta[c]	β [95% CI]	Std. Beta[c]
Cord blood zinc	0.29 [−1.24, 1.82] $p = 0.702$	0.052	−0.23 [−0.06, 0.01] $p = 0.532$	−0.212	−0.04 [−0.22, 0.14] $p = 0.620$	−0.065	0.13 [−0.19, 0.45] $p = 0.418$	0.118
Cumulative zinc intake (mg)	2.139 [0.48, 3.80] $p = 0.013^{*}$	0.339	0.01 [−0.02, 0.05] $p = 0.532$	0.090	0.08 [−0.12, 0.28] $p = 0.426$	0.102	0.40 [0.06, 0.75] $p = 0.024^{*}$	0.331
Age at the end of study (days)	−0.36 [−1.86, 1.15] $p = 0.635$	−0.063	−0.01 [−0.04, 0.02] $p = 0.567$	−0.083	NA		NA	
Birth weight (g)	−0.86 [−2.38, 0.66] $p = 0.261$	−0.155	−0.03 [−0.06, 0.01] $p = 0.125$	−0.234	0.36 [0.18, 0.53] $p < 0.001^{*}$	0.533	0.20 [−0.11, 0.51] $p = 0.198$	0.187
Infant gender[d]	−4.79 [−7.82, −1.76] $p = 0.003^{*}$	−3.185	−0.07 [−0.13, −0.01] $p = 0.036^{*}$	−0.310	NA		NA	
Maternal BMI	0.78 [−0.68, 2.25] $p = 0.287$	0.144	0.01 [−0.01, 0.05] $p = 0.363$	0.134	−0.03 [−0.20, 0.14] $p = 0.701$	−0.049	0.20 [−0.10, 0.50] $p = 0.185$	0.191

Note. Independent variables were transformed to SD score before entering the regression model.
Abbreviations: LAZ, length-for-age Z-score; WLZ, weight-for-length Z-score.
[a]Infants' growth parameters were used as dependent variables. The models for total weight and length gain were adjusted by infant age, birth weight, infant sex and maternal BMI. The models for LAZ and WLZ were adjusted by birth weight and maternal BMI.
[b]Total weight and length gain from birth to 4 months.
[c]Standardized beta coefficient.
[d]Infant gender: male = 1; female = 2.
$^{*}p < 0.05$.

TABLE 5 Associations between cord blood ferritin and cumulative iron intake from 0 to 4 months with infant's growth and nutritional status at 4 months

Variables (SD score)	Growth parameters[a] Total weight gain (g)[b] β [95% CI]	Std. Beta[c]	Total length gain (cm)[b] β [95% CI]	Std. Beta[c]	LAZ β [95% CI]	Std. Beta[c]	WLZ β [95% CI]	Std. Beta[c]
Cord blood ferritin	−1.50 [−3.22, 0.22] $p = 0.085$	−0.267	0.02 [−0.02, 0.05] $p = 0.401$	0.138	−0.04 [−0.23, 0.15] $p = 0.683$	−0.057	−0.31 [−0.66, 0.03] $p = 0.074$	−0.289
Cumulative iron intake (mg)	−0.34 [−1.91, 1.23] $p = 0.663$	−0.062	0.01 [−0.03, 0.04] $p = 0.746$	0.030	−0.11 [−0.29, 0.07] $p = 0.237$	−0.160	0.02 [−0.31, 0.34] $p = 0.926$	0.014
Age at the end of study (days)	−0.44 [−2.33, 1.44] $p = 0.638$	−0.065	−0.02 [−0.06, 0.02] $p = 0.433$	−0.116	NA		NA	
Birth weight (g)	−1.23 [−2.77, 0.32] $p = 0.118$	−0.219	−0.02 [−0.05, 0.02] $p = 0.296$	−0.156	0.37 [0.20, 0.55] $p < 0.001^*$	0.547	0.10 [−0.21, 0.41] $p = 0.523$	0.093
Infant gender[d]	−4.48 [−7.77, −1.18] $p = 0.009^*$	−0.386	−0.08 [−0.15, −0.01] $p = 0.022^*$	−0.361	NA		NA	
Maternal BMI	−0.14 [−1.82, 1.54] $p = 0.869$	−0.166	0.03 [−0.01, 0.06] $p = 0.158$	0.225	−0.06 [−0.25, 0.12] $p = 0.509$	−0.088	0.02 [−0.31, 0.36] $p = 0.887$	0.022

Note. Independent variables were transformed to SD score before entering the regression model.

Abbreviations: LAZ, length-for-age Z-score; WLZ, weight-for-length Z-score.

[a]Infants' growth parameters were used as dependent variables. The models for total weight and length gain were adjusted by infant age, birth weight, infant sex and maternal BMI. The models for LAZ and WLZ were adjusted by birth weight and maternal BMI.

[b]Total weight and length gain from birth to 4 months.

[c]Standardized beta coefficient.

[d]Infant gender: male = 1; female = 2.

$^*p < 0.05$.

the achieved infant weight (WLZ) at age 4 months, and with weight gain from birth to the age of 4 months. We found a significant positive association between iron storage and iron status measured by serum ferritin at 4 month of age. To our knowledge, this is the first study in Thailand describing zinc and iron intakes in breastfed infants and the association of nutrient stores at birth and nutrient intakes among breastfed infant with their subsequent nutrient status and growth.

The volume of breast milk reported in our study is similar to previous studies determining breast milk intakes among breastfed infants using the deuterium oxide technique in Thailand (Tongchom et al., 2020) and other developing countries (Bandara et al., 2015; Daniels et al., 2019). Mean breast milk intake at 4 months was significantly higher than at 2 months, whereas the breast milk intake per kg infant bodyweight was lower at 4 than 2 months. It was possible that due to a lower growth velocity, hence, lower energy requirement per kg body weight at 4 months. This finding is similar to that found among Indonesian infants, showing an inverse correlation between infant age and breast milk intake per kg body weight (Daniels et al., 2019).

Daily zinc intake from breast milk in our study was higher than data reported from Indonesia (Daniels et al., 2019), and South India (Samuel et al., 2014) at similar infant ages. With the decline in zinc available from breast milk, the prevalence of inadequate zinc intake was increased from 2 months to a rather high level of 40% at 4 months of age. The decline in total zinc intake from 2 to 4 months occurred even though infants consumed a higher breast milk volume at 4 than 2 months. Little is known on the factors associated with the decline of BMZn with increasing duration of lactation. A previous randomized controlled trial study in Egypt showed that a decrease in breast milk zinc from birth to 2 months was smaller in lactating women receiving a daily 10-mg zinc supplement compared with placebo, implying that maternal zinc intake might influence the zinc content in breast milk (Shaaban et al., 2005). In this study, there was no difference in declined BMZn between women who used supplement and who did not, but we found that women with higher maternal body fat percentage had lesser reduction in BMZn, among the study population with normal nutritional status (mean body fat percentage and BMI were 31.5 ± 7.7% and 22.9 ± 3.6 kg/m^2, respectively). To our knowledge, there was no previous study showing this association. Future studies are needed to clarify the effect of maternal body composition on reduction of BMZn.

In contrast to zinc, iron concentration in breast milk was relatively stable, but also highly variable, over time. Infants received comparable amounts of daily iron intake from breast milk at both ages (2 and 4 months). Iron intakes of breastfed infants in our study were higher than previously reported among Indonesian infants (Daniels et al., 2019). The prevalence of iron deficiency in our study population was much lower than that of zinc deficiency, with about 11% of infants considered iron deficient at 4 months of age.

The relative importance of neonatal iron storage on iron status at 4 months was also found in the presence study (Table 3), indicating the criticality of adequate iron accretion during fetal life. A study from China also reported that cord blood iron profiles, measured as cord blood haemoglobin, zinc protoporphyrin/heme and serum transferrin saturation, were related to iron status of infants even at the age of 9 months (Shao et al., 2021). Cord blood ferritin is a major predictor of iron status during the first few months postnatal, as they provide generally adequate amounts of iron to cover the needs for utilization during up to 6 months in healthy term infants (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2015). This may explain why the relatively low concentrations of BMFe have little effect on iron status among breastfed infants during the first months after birth (Pérez-Escamilla et al., 2019).

We found cumulative zinc intakes positively associated with growth in weight (total weight gain and WLZ) at 4 months of age, but not with length. A study from South India reported no association between average daily zinc intakes from breast milk and both infant weight and length gain during the first 6 months (Samuel et al., 2014). A meta-analysis showed that zinc supplements had positive effects on WAZ and WLZ, but not LAZ of infants under 6 months of age (Lassi et al., 2020). Zinc supplementation was shown to positively affect weight parameters of infants and young children under 2 years of age. Among children older than 2 years, the effect on height was greater (Liu et al., 2018). Therefore, zinc might be related with lean mass and weight parameters than linear growth during early infancy period. Cord blood zinc had no association with any of growth parameters determined at 4 months, and was of relatively less importance compared to cumulative zinc intake when considered the effect size (standardized beta coefficient) on growth (Table 4). Better understanding of the relative contribution between zinc storage or endowment from fetal life versus adequacy of intakes, especially from breast milk during early infancy (till 6 months, if considered exclusive breast feeding) needs further elucidation. Our study did not find the association of zinc storage or intakes with infant serum zinc. Accretion of zinc and intake from breast milk may be related with body zinc or zinc metabolism in infant's body. However, the limitation of using serum zinc as a biomarker for zinc status should take into account.

Strengths of our study are the prospective design with inclusion of women in a developing country, a strictly standardized study protocol, including measurement of breast milk volumes in individuals, which together enables us to provide reliable data on zinc and iron intakes in individual breastfed infants. There are some limitations in our study. Inflammation may affect serum zinc and ferritin. Our study did not have the information of inflammatory marker, but we screened the participants by history of previous illness during 2 weeks before data collection. While it was planned to use the best method of estimating human milk intake using stable isotope method, we could not apply this technique to all study participants. We had to rely on the mothers/caretakers records of weighing amount of breast milk taken by infants to derive the daily intake, since some women had to return to work and could only provide expressed breast milk for their infants. Another limitation in our study is the withdrawal of almost 50% of enrolled study participants by 4 months postpartum, resulting in much smaller sample size than originally planned. This sample size issue might be most critical in examining the association and relative importance of sources of zinc and iron on biochemical status and growth. The drop-outs included lactating women who had stopped breastfeeding due to lack of breastfeeding support, separation from their babies when returning to work, reported inadequate milk production.

5 | CONCLUSION

This study provides quantitative data on the daily zinc and iron intakes of breastfed infants. Zinc intakes among breastfed infants both at 2 and 4 months of age were found to be inadequate, and accordingly, the prevalence of zinc deficiency at 4 months of age was quite high (76%). In contrast, iron intake from breast milk appeared to be adequate at both ages. However, the prevalence of iron deficiency was found in 11% of infants at age of 4 month. The relative importance of zinc/iron storage versus intakes for these breastfed infants was examined on biochemical status and growth at 4 months of age. Zinc intake was associated with infant weight gain and achieved weight at 4 months of age. Iron storage measured by ferritin concentration in cord blood at birth was more important than iron intake from breast milk on iron status, but not growth at 4 months of age.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

All authors contributed to the study design and construction of research protocol. The data collection process was conducted by OD. Data analysis was performed by OD and PW. OD drafted the manuscript and revised by PW and BK. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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3. Conclusion

This study quantified zinc and iron intakes among breastfed infants at the age of 2 and 4 months. We found high proportion of infants with inadequate zinc intake and high prevalence of zinc deficiency among 4-month-old breastfed infants. Breast milk zinc intake was positively associated with infant weight parameters, but iron intake was not associated with either serum ferritin or infant growth parameters. Instead, we found iron store had more contribution to iron status of breastfed infant at 4 months old.

Appendix

Publication of a study protocol

Dumrongwongsiri O, Winichagoon P, Chongviriyaphan N, Suthutvoravut U, Grote V, Koletzko B. Determining the Actual Zinc and Iron Intakes in Breastfed Infants: Protocol for a Longitudinal Observational Study. JMIR Res Protoc. 2020 Nov 6;9(11):e19119. doi: 10.2196/19119

Protocol

Determining the Actual Zinc and Iron Intakes in Breastfed Infants: Protocol for a Longitudinal Observational Study

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Abstract

Background: Zinc and iron deficiencies among breastfed infants during the first 6 months of life have been reported in previous studies. The amounts of zinc and iron intakes from breast milk are factors that contribute to the zinc and iron status of breastfed infants.

Objective: This study aims to quantitatively determine zinc and iron intakes by breastfed infants during the first 4 months of life and to investigate the factors that predict zinc and iron status in breastfed infants.

Methods: Pregnant women at 28 to 34 weeks of gestation were enrolled. Zinc and iron status during pregnancy was assessed. At delivery, cord blood was analyzed for zinc and iron levels. Participants and their babies were followed at 2 and 4 months postpartum. Maternal dietary intakes and anthropometric measurements were performed. The amount of breast milk intake was assessed using the deuterium oxide dose-to-mother technique. Breast milk samples were collected for determination of zinc and iron levels. The amount of zinc and iron consumed by infants was calculated. Zinc and iron status was determined in mothers and infants at 4 months postpartum.

Results: A total of 120 pregnant women were enrolled, and 80 mother-infant pairs completed the study (56 provided full breastfeeding, and 24 provided breast milk with infant formula). All data are being managed and cleaned. Statistical analysis will be done.

Conclusions: This study will provide information on zinc and iron intakes in exclusively breastfed infants during the first 4 months of life and explore predictive factors and the possible association of zinc and iron intakes with infant growth and nutrient status.

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KEYWORDS

breastfeeding; zinc; iron; zinc deficiency; iron deficiency; deuterium oxide dose-to-mother technique; infant; baby; diet; protocol; prediction; women; growth

Introduction

Micronutrients are essential for infant growth and development. During the first 6 months of life, infants obtain micronutrients from breast milk if they are exclusively breastfed, as recommended by the World Health Organization (WHO) [1]. In addition to micronutrients provided by breast milk, infants use body stores of micronutrients deposited during pregnancy.

Zinc and iron are particularly important micronutrients for infants. Some previous studies have shown that zinc and iron deficiencies are associated with delayed infant growth and development, especially when such deficiencies occur during the early period of life [2-4]. Several reports have shown zinc and iron deficiencies in a high proportion of infants younger than 6 months, and breastfeeding was found to be the associated factor. A study in 2007 in the northeast area of Thailand reported that 50.8% of breastfed infants aged 4 months had a zinc deficiency [5]. When comparing the prevalence of zinc deficiency by feeding types, zinc deficiency was more prevalent among 4- to 6-month old breastfed infants compared with formula-fed infants (14.9% and 5.3% among breastfed and formula-fed infants, respectively) [6]. Regarding iron deficiency, the prevalence of iron deficiency among breastfed infants was found to be higher than among infants fed with formula in several studies [7,8]. A study on the iron status of infants in Bangkok showed that the prevalence of iron deficiency anemia in breastfed infants aged 1 year was 25.7%, which was higher than in formula-fed infants (2.7%) [9]. The percentage of infants with iron deficiency anemia was 4 times higher in 6-month-old compared with 4-month-old infants (26.1% vs 5.7%, respectively), as described in a cohort study of iron status in breastfed infants [10]. Breastfeeding duration was found to be associated with a higher prevalence of iron deficiency [11] and low serum ferritin or other iron markers among infants and children [12,13].

The zinc and iron status of breastfed infants during breastfeeding is associated with several factors. The amount of iron storage during intrauterine life, zinc and iron intakes, and physiologic requirement are proposed to be the factors determining zinc and iron status of breastfed infants [14]. Infants' iron storage depends on maternal nutrient status during pregnancy and can be observed in cord blood levels [15,16]. Daily zinc and iron intakes of exclusively breastfed infants come from zinc and iron in breast milk. Naturally, micronutrient concentrations in breast milk are not constant but dynamically change during lactation. Zinc and iron concentrations in breast milk are high during early lactation and gradually decline thereafter [17-19]. The amounts of zinc and iron in breast milk consumed by infants after 6 months are lower than the estimated daily requirements [20,21]. The majority of zinc and iron intakes in infants during this period need to be provided by complementary foods.

Several hypotheses have been proposed to explain the causes of zinc and iron deficiencies among breastfed infants during the exclusive breastfeeding period. Low micronutrient concentrations in breast milk have been proposed as a factor associated with nutrient deficiency in breastfed infants. Stronger evidence was shown in the case of zinc deficiency compared with iron deficiency [22]. Recent studies have attempted to explore the factors that might be associated with the low micronutrient concentrations in breast milk. There have been reports on genetic variation of zinc transporters resulting in the difference in breast milk zinc concentrations [23,24]. Some studies have reported that socioeconomic status, maternal dietary intake, maternal anthropometric parameters, micronutrient status, and maternal age are associated with zinc and iron concentrations in breast milk [6,25,26]. However, many studies did not confirm these associations [27,28].

Breast milk provides complete nutrition to infants during the first 6 months of life, but zinc and iron deficiencies occur among breastfed infants. While zinc and iron levels in breast milk have been determined and reported in several studies, they do not directly reflect the zinc and iron intake amounts in breastfed infants. The data on breast milk volume taken by infants and the nutrient levels in breast milk better demonstrate the amounts of zinc and iron taken by breastfed infants. However, the measurement of breast milk volume consumed by infants can be challenging. Traditional assessments using the test-weighing method or the measurement of expressed breast milk have considerable inaccuracies. Stable isotope measurement of breast milk intake with the protocol established by the International Atomic Energy Agency (IAEA) is the most accurate method to quantify the infant's breast milk intake [29].

Zinc and iron intake from breast milk is one of the factors determining the zinc and iron status of breastfed infants. The data on nutrient intakes will provide more information regarding the zinc and iron status of breastfed infants and may lead to the prevention of nutrient deficiencies. Our study aims to quantify zinc and iron intakes by measuring micronutrient levels in breast milk and assessing breast milk volume intake by breastfed infants using the deuterium oxide dose-to-mother technique.

Methods

Recruitment

This is a prospective descriptive study at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The study protocol was approved by the human research ethics committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 03-60-31) and the ethical committee of Ludwig Maximillian Universitaet, Munich (Project No. 18-015). Pregnant women visiting the antenatal care (ANC) clinic at Ramathibodi Hospital at 28 to 34 weeks of gestation were eligible for enrollment. The enrollment was performed at the ANC clinic when the pregnant women attended the education class during their second trimester. Inclusion criteria were healthy pregnant women who planned to deliver their babies at Ramathibodi Hospital, intended to breastfeed their babies at least 4 months, lived in the Bangkok metropolitan area, and provided written informed consent. Pregnant women who carried twin or triplet pregnancies or who had any contraindication for breastfeeding were excluded. Women and their babies were followed until 4 months postpartum. Each participant was invited to 4 visits (ie, at enrollment, at delivery, and at 2- and 4-month postpartum). The details of the data collection are summarized in Figure 1. During the study, women who were unwilling to participate in the study, stopped breastfeeding, moved to another province, or had babies with chronic diseases or serious illness were excluded from continuing study participation. The recruitment period was from March 2018 to September 2019.

Figure 1. Data collected at each visit in the study.

Data collection	Enrollment	Delivery	2 months post partum	4 months post partum
Demographic data	√			
Perinatal data		√		
Assessment of dietary intake	√		√	√
Assessment of body composition in lactating women			√	√
Infant growth assessment			√	√
Blood sample for zinc and iron status	√ (mother)	√ (cord blood)		√ (mother and infant)
Breast milk sample for zinc and iron levels			√	√
Measurement of breast milk intake volume			√	√

Sample Size Calculation

Sample size calculation was based on the reported mean zinc intake of breastfed infants in a study by Krebs et al [30], which was 1.00 (SD 0.43) mg/day. The sample size was determined using the 1-sample *t* test for mean formula, as follows:

$$n = \left[\frac{\sigma}{\mu 1-\mu 0}\right]^2 \left[Z_{\alpha/2} + Z_{(1-\beta)}\right]^2 \quad \textbf{(1)}$$

Using the assumption that this study will provide a difference in the mean of 0.15 from the previous study, and given a significance level of .05 and power of 0.8, the calculated sample size of this study was 64:

$$n = \left[\frac{0.43}{0.15}\right]^2 [1.96 + 0.84]^2 \quad \textbf{(2)}$$

n=64

The calculated sample size of this study was 64. We estimated that the dropout rate would be up to 30%. Therefore, the calculated sample size was 100 participants:

$$\text{Sample size} = \frac{64}{1\text{-}0.3} = 91 \sim 100 \quad \textbf{(3)}$$

Data Collection

Demographic Data and Antenatal Data

Demographic data, including maternal age, existing diseases, education level, and socioeconomic status, were obtained by interviewing participants. Antenatal data were retrospectively reviewed from medical records and the maternal pregnancy handbook. Data collected during pregnancy included prepregnant weight and BMI, weight gain during pregnancy, parity, investigations during antenatal care (every pregnant woman had a blood test for anemia and serologic screening during their first visit to the ANC clinic and some had an oral glucose tolerance test to screen for gestational diabetes, depending on clinical indication), and complications during pregnancy (ie, gestational diabetes, preeclampsia, and others).

Perinatal Data

The investigators visited the participants who delivered their babies at Ramathibodi Hospital at the postpartum ward. Mothers received routine postpartum care and education from the ward staff. Data regarding mode of delivery, delivery complications, and perinatal complications of infants were collected. Infant anthropometric data, including birth weight, length, and head circumference, were routinely measured by nurses in the labor room.

Anthropometric Assessment of Lactating Women and Infants

To determine the nutritional status of lactating women and infants, anthropometric measurements were performed at the 2- and 4-month postpartum visits. For lactating woman, weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest 0.1 cm using a height scale while the woman was standing upright without shoes or hair ornaments. Weight, BMI, fat mass, skeletal muscle mass, and visceral fat area were measured using a body composition analyzer (InBody 720; InBody Co).

For the infant, weight was measured to the nearest 10 grams using a digital baby scale, and recumbent length was measured to the nearest 0.1 cm using a wooden board with a sliding foot piece. Head circumference was determined using a nonstretchable measuring tape. The occipitofrontal circumference was measured twice; the greater value was used to represent the baby's head circumference. Weight, length, and head circumference were calculated to *z* score for age and sex, according to the WHO growth chart from the WHO Anthro calculator. In addition to the anthropometric measurements, the weight and length gain of the infants was calculated to determine the growth rate during the first 4 months.

Assessment of Maternal Dietary Intakes

Dietary intake was important during pregnancy and lactation. There are a lot of factors influencing maternal food intake during these periods, such as beliefs and traditions, lifestyle, anxiety, socioeconomic status, and family support. We assessed maternal dietary intakes during pregnancy (at enrollment) and lactation (at 2- and 4-month postpartum) using 3 dietary intake assessment tools, namely a 24-hour food recall, the food frequency questionnaire (FFQ), and a 3-day prospective dietary record. The FFQ was constructed to determine zinc and iron intake with common foods eaten by Thai people. At participant visits, dietary history (24-hour food recall and FFQ) was recorded by a skilled dietitian or nutritionist. A 3-day food record form was then handed to the participant to complete at home within 2 weeks after the visit. They were asked to send the food record back to the researcher by mail or to bring it back at the next visit. The amounts of nutrient intake, including energy, protein, zinc, and iron, were analyzed using INMUCAL software version 4.0 (Institute of Nutrition, Mahidol University), which is the largest database of nutrients in Thai foods.

Collection and Analysis of Blood Samples

Blood samples for determining zinc and iron status were collected. Maternal blood samples were collected from an antecubital vein during pregnancy (at enrollment) and lactation (at 4-month postpartum). At delivery, cord blood samples were collected right after cord cutting from the umbilical cord on the placental side. Infant venous blood samples were collected at the age of 4 months.

Blood samples were immediately centrifuged to separate plasma and kept frozen at –80 °C. All containers used for sample collection were washed with a nitric acid solution and deionized water in order to avoid contamination with micronutrients from the environment. Zinc concentration was analyzed using flame atomic absorption spectrophotometry (GBC Avanta S; GBC Scientific Equipment). Serum ferritin and complete blood count were analyzed using chemiluminescence (automated) and electrical impedance, respectively, at the Department of Pathology, Faculty of Medicine Ramathibodi Hospital. Remaining plasma samples were kept for further analysis.

Collection and Analysis of Breast Milk

Each lactating woman was asked to collect a breast milk sample at the 2- and 4-month visits. Breast milk samples were collected from one breast by an electrical milk pump. The participant was asked to express breast milk until the breast was empty. The breast milk sample was then evenly mixed, and 15 mL of the breast milk sample was collected for analysis. The remaining breast milk was kept in the milk storage bag and returned to the participant for feeding her infant. The milk samples were kept at –80 °C within 4 hours from the time of collection. All the equipment and containers used in breast milk collection had been washed with a nitric acid solution and deionized water to avoid micronutrient contamination from the environment and were sterilized before use. A separate aliquot of 5 mL of the evenly mixed breast milk sample was transferred for metabolomics analyses.

Zinc and iron levels in the breast milk were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). Prior to analysis by ICP-OES, the breast milk sample was digested using nitric acid in a closed vessel under microwave radiation.

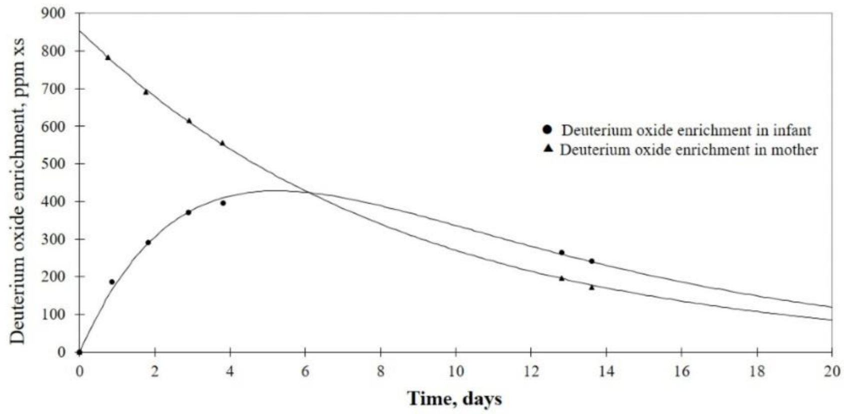
Measurement of Breast Milk Volume Intake by Infants Using Deuterium Oxide Dose-to-Mother Method

The breast milk volume was assessed at 2- and 4-month postpartum. In general practice, there are two methods of giving breast milk to infants: providing breastfeeding at the breast or expressing breast milk via bottle-feeding. We used different methods to assess breast milk volume from different feeding practices.

Among mothers who provide breastfeeding at the breast, breast milk intake was assessed using the deuterium oxide dose-to-mother technique, strictly following the protocol to assess breast milk intake proposed by the IAEA [29]. Deuterium is a stable (nonradioactive) isotope of hydrogen that is metabolized in the body in the same way as water. Therefore, deuterium oxide is eliminated from the body in urine, saliva, sweat, and human milk.

The principle of the deuterium oxide dose-to-mother technique for measuring breast milk volume is to track the disappearance of deuterium oxide from the maternal body and the presence of deuterium oxide in the infant (Figure 2). Lactating women were given a drink of 30 g of deuterium-labeled water. Saliva samples (2 mL per sample) were collected from mothers and infants to monitor deuterium oxide levels. According to the IAEA protocol, saliva samples were collected at 7 time points: at baseline (day 0) before giving the deuterium-labeled water to the mother and at days 1, 2, 3, 4, 13, and 14 after the dose of deuterium. All samples were collected by the same researchers, both at the hospital (on day 0) and during home visits. Deuterium oxide levels were analyzed and breast milk volumes were calculated using the equation based on the principles of volume distribution [29].

Figure 2. Deuterium oxide enrichment from the deuterium oxide dose-to-mother technique to measure breast milk intake shows the disappearance of deuterium oxide from the mother's body and the presence of deuterium oxide in the infant. xs: excess.



Results

Study enrollment took place from March 2018 to September 2019. A total of 120 pregnant women participated in this study. There were 3 participants who delivered their babies in other hospitals and were excluded from the study. Among the 117 participants, 56 women provided breastfeeding to their babies and completed the study at 4-month postpartum; they were classified as the breastfeeding group. A total of 24 women could not adequately provide breastfeeding to their babies and gave the infants some infant formula. However, they completed the study and were classified as the mixed-feeding group. A total of 37 women were excluded from the study (18 stopped breastfeeding before 4 months, 12 moved to another province, and 7 were unwilling to continue the study). All data are being managed and cleaned. Statistical analysis will be done.

Discussion

Expected Outcome

This study will provide information on zinc and iron intakes from breast milk in breastfed infants during the first 4 months of life. It may demonstrate the association of zinc and iron intakes with the growth and nutrient status of infants. As this study follows the participants from pregnancy to lactation, the data may provide information about the impact of intrauterine nutrition on the nutrient status of infants after birth. The data regarding dietary intake and nutrient status of mothers during both the pregnancy and lactation period will be provided and the relationship between maternal and infant nutritional status may be demonstrated.

Significance of the Study

This study will provide informative data on zinc and iron intakes by breastfed infants. These data will provide scientific knowledge and might contribute to determining the daily dietary zinc and iron requirements for infants during the first 6 months. Moreover, these data may be useful in devising strategies for preventing zinc and iron deficiency in breastfed infants. As the study will also provide information on the levels of zinc and iron in breast milk and their associations with the dietary intake and micronutrient status of lactating women, these data will have advantages for nutritional promotion during lactation.

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Conflicts of Interest

None declared.

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Abbreviations

ANC: antenatal care
FFQ: food frequency questionnaire
IAEA: International Atomic Energy Agency
ICP-OES: inductively coupled plasma optical emission spectrometry
WHO: World Health Organization

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