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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).

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.

**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.

## 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

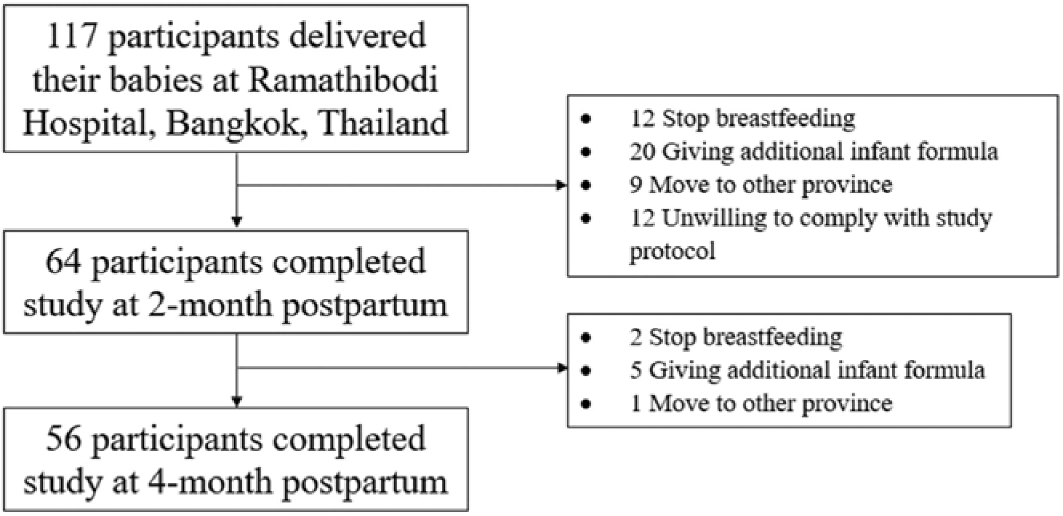

**FIGURE 1** Participants in the study

**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 (β = −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 (β = −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

| | Growth parameters[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total weight gain (g)[b] | | Total length gain (cm)[b] | | LAZ | | WLZ | |
| Variables (SD score) | 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

| | Growth parameters[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total weight gain (g)[b] | | Total length gain (cm)[b] | | LAZ | | WLZ | |
| Variables (SD score) | 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 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 dropouts 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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