Longitudinal study on breastfeeding and growth for 0-6 month infants

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ABSTRACT

Background and Objectives: To explore advantages and challenges for exclusive breastfeeding (EBF), compared to non-exclusive breastfeeding (nEBF). **Methods and Study Design:** Mothers were enrolled from 7 cities in China, and were visited at 3, 10, 60, 120, and 180 days postpartum. Data about feeding practices, infant growth, and the macronutrient contents of human milk (HM) were collected. Weight-for-age (WAZ), length-for-age (LAZ) and weight-for-length (WLZ) z scores were calculated. **Results:** 130 lactating mothers attended 5 visits. 59 mothers (45.4%) exclusively breastfed infants for 0-4 month; 71 mothers (54.6%) gave formula to their infants. Frequencies of breastfeeding per day were higher in the EBF group than the nEBF group at day 3, 10, 120 and 180, and were less than 8 times per day in the nEBF group at all time points. For WAZ, there were no differences between the two groups. LAZ was greater in the nEBF group at day 180 (0.74+1.05 vs 0.33+1.28). WLZ were greater in the EBF group than the nEBF group at day 120 and 180 (day 120: 0.88+1.08 vs. 0.36+1.1, day 180: 1.1+0.94 vs 0.54+1.07). The average protein and lactose contents of HM in the nEBF group were higher than in the EBF group at day 10. **Conclusions:** For nEBF infants, intake of formula replaced intake of breastmilk, due to lack of breastfeeding frequency, which did not bring weight gain for nEBF infants. During the introduction of complementary foods, EBF infants needed complementary nutrients to support growth. Breastfeeding practice was indirectly related to HM macronutrient content. Therefore, lactating mothers may need to provide appropriate complementary feeding and maternal leave extension to attend to their infant's nutritional requirements. The criteria for linear growth may also need to be more commensurate with breastfeeding and relevant to later health outcomes.

Key Words: exclusive breastfeeding, mixed feeding, infant growth, human milk composition, longitudinal study

INTRODUCTION

Human milk (HM) is the optimal food for infant because it provides energy, protein, micronutrients and bioactive components. Breastfeeding has short-term benefits on infant health, particularly reducing diarrhea and respiratory infection; also has long-term beneficial effects on infant health and development, such as reducing overweight and obesity, improving intelligence development.[1,2]

In 2001, World Health Organization (WHO) reviewed the optimal duration of exclusive breastfeeding (EBF), concluded that EBF for the first 6 months of life had no observable deficits for infant growth in both developing and developed countries.[3] In 2004, WHO Global Strategy recommended that infants should be exclusively breastfed for the first 6 months of life.[4] Many low- and middle-income countries incorporated this recommendation into national health policies and child survival programs.[5] However, rate of EBF was still low, and only 39% of infants were exclusively breastfed for 6 months globally in 2010.[6] It is a worldwide challenge of exclusively breastfeeding the infants for 6 months.[5] The determinates of these rates varied from low- to high-income countries: in low-income countries included cultural beliefs, education, marketing of formula, and access to healthcare; in high-income countries included obesity, returning to work, poor family support, and education.[7] In China, a national report disclosed that the rate of EBF in 0-6-month infants was 28% in 2008.[8] Although the infant feeding strategy of China was issued in 2005, which also recommended infants be exclusively breastfed for 6 months, lots of challenges still exist, especially lack of community supporting services.

The awareness of breastfeeding among mothers has risen, and the need for services to provide breastfeeding guidance has increased. If those services are not available, mothers will turn to substitute feeding practice for help. Such services might include breastfeeding consulting, and infant growth surveillance in nutrition clinic. Besides, the determination of HM major composition, such as fat, protein and lactose, which provide the majority of the energy in HM, has been a topic of extensive research among various populations around the world and is becoming more widely available in clinics of China.[9,10] The use of rapid HM analysis machines, avoid the time-consuming use of separate analytical instruments and can provide instant feedback to mothers regarding their milk composition.[11] Such feedback can be useful for supporting breastfeeding. When combined with infant HM intake information, information about the macronutrient contents of HM can provide information about the infant's energy intake.[12]

In the past decades, there were numerous studies conducted to explore EBF 6 months and infant health. However, there have been seldom longitudinal studies on EBF and infant growth reported from China.[13] Therefore, it would be very important to conduct longitudinal study on tracing growth of EBF infants and combining HM analysis to support breastfeeding and to achieve optimal development of infant.

MATERIALS AND METHODS

Subjects

Mothers from 7 cities in China (Jiamusi, Beijing, Ji'nan and Xi'an in the northern China; Xinmi, Kunshan and Chengdu in the southern China) were enrolled in the collection of longitudinal data. The study was carried out from January 2013 to June 2014.

The inclusion criteria for the participants were : (1) healthy mothers without diseases thataffect lactation, including infectious diseases and any severe disease; (2) mothers aged between 20 and 40 years old during pregnancy; (3) mothers who were non-smokers and non-drinkers; (4) infants born at term;(5) infants born without congenital diseases; (6) mothers who planned exclusive breastfeeding for 6 months.

The Ethics Committee of the Capital Institute of Paediatrics approved the study according to the International Organizations of Medical Sciences' "Human biomedical research international guidelines" and the ethical principles from the Declaration of Helsinki. We obtained written informed consent from the lactating mothers and on behalf of the infants involved in this study. Advice regarding infant feeding and health was provided to the subjects free of charge.

Data collection

Considering the alteration of breastmilk compositions (colostrum: 1-7 postpartum days; transitional breastmilk: 7-14 days; mature breastmilk: >14 days) and the feasibility of child growth survey, longitudinal observation was conducted at 3, 10, 60, 120, and 180 days postpartum. Demographics, infant growth information, feeding practices, and HM for macronutrient analysis were collected. Weights and lengths of the infants were measured by trained paediatric research nurses. Infant feeding practices, including frequency of breastfeeding per day (FBF), average sucking time per breastfeeding session (SBF, minute) and any other food and drink in the past 24 hours were recorded. Total breastfeeding time per day (TBF, minute) was equal to the value of multiplying the SBF by the FBF. General health information for the mothers and infants was surveyed using questionnaires with detailed questions about the mother's age, education level, and occupation and the infant's gender and birth condition.

Milk samples were analyzed by the Human Milk Analyzer (HMA-2000) produced by Hongyang corporation of China. This device uses a simple and rapid method of ultrasound transmission spectroscopy to measure fat, protein and lactose content of HM. By measuring sound velocity, acoustic attenuation, acoustic impedance and the coefficient of adiabatic compressibility, the machine establishes mathematic models for each macronutrient. The energy in HM was calculated based on its fat, protein and lactose contents. The HM samples were collected manually from one breast between 9:00-11:00am. 5 mL of breast milk were collected in the middle of a meal (3-5 minutes after the infant starting sucking), which were stored at the room temperature (16°C) and tested 6 hours after collection.

Compared with traditional experimental methods (the infrared spectroscopy method for protein, the modified Gravimetric method for fat, and high-performance liquid chromatography for lactose) for measuring the nutritional content of mature HM and formula, the average macronutrient content determined with the HMA deviated less than l%. Furthermore, the HMA method had good reproducibility (relative standard deviation <4%) and a high recovery rate (95-99%).[9]

Quality control included: (1) staff training: all the surveyors were trained on the process of collecting health data, surveying and recording; all the operational staff were trained on collecting and analysing HM according to the program plan; (2) data quality monitoring: A supervisor in each city monitored data quality throughout the data collection processes to find missing and incorrect data and correct them; (3) data input and check: A specific internet-based data input system was used, and the data inputters checked and corrected the data before entering them, and the data were scrutinized and logically corrected after they were entered.

Data analysis

The data were analysed by the SPSS statistical software (Version 20.0 for Windows). The distributions of study variables were examined to test for non-normal distributions, outliers, and missing data. Numerical variables are presented as the means (M) and standard deviations (SD) for distribution, and categorical variables are presented as the proportions. T-tests and chi-square tests were used to investigate the differences between two groups (EBF vs nEBF). Considering that the infant growth data were comparable for different ages and genders, the weight and length data were converted to z scores using WHO Anthro program (version 3.2.2, 2011). The z-scores of weights for age (WAZ), length for age (LAZ) and weight for length (WLZ) were calculated. Multilevel models were used for the analysis of associations between EBF and breastfeeding practice, infant growth, HM macronutrients contents, as well as potential confounders. Multivariate linear regression model was used, in which FBF, SBF, TBF, WAZ, LAZ, WLZ, and HM macronutrients (fat, protein, lactose and energy) contents were modelled as dependent variables separately, and breastfeeding practice, sex, birth weight, city, birth way, enrolling season, maternal age, maternal occupation and maternal education level were put into models as independent variables. Breastfeeding practices were divided into two groups: EBF (the value was '1') and nEBF (the value was '0'), the other factors were considered as confounders. $p<0.05$ was considered statistically significant.

RESULTS

General information

A total of 130 lactating mothers in the longitudinal team had finished all 5 visits. 59 mothers (45.4%) exclusively breastfed their infants for 0-4 months, and 71 mothers (54.6%) had added formula to feed their infants. Therefore, Mothers and infants are divided into two groups: exclusive breastfeeding (EBF) group and non-exclusive breastfeeding (nEBF) group. The characteristics of the participants is listed in Table 1 and Table 2, which provide proportions and means. Over 59% of the mothers in our study had a college education and 41.4% of mothers were housewives. Two groups (EBF vs nEBF) were comparable for the general information. There were no significantly differences in gender, birth way, birth length, birth weight, maternal occupation, maternal prenatal BMI and maternal age between two groups, except regional distribution and maternal educational levels. The EBF group was mainly from the southern China; the nEBF group was mainly from the northern China. Mothers in the EBF group had higher education level than in the nEBF group.

Feeding practices

Although mothers are divided into two groups based on the feeding practices in the first 4 months, mothers in the nEBF group did not always use formula at each postpartum time. As shown in Figure 1, 59 mothers added formula at day 3, and then 26, 20, 17 mothers used formula at day 10, 60, 120 respectively. At day 180, total 126 mothers (96.9%) added complementary food to their infants.

As shown in Figure 2, for t-tests, average FBF at day 3, 10, 120 and 180 were significantly higher in the EBF group than in the nEBF group (day 3: 8.25+2.09 vs 6.54+3.06, $p<0.01$; day 10: 8.49+1.19 vs 7.84+2.00, $p=0.02$; day 120: 7.80+1.14 vs 7.21+1.61, $p=0.02$; day 180: 6.95+1.66 vs 6.30+2.95, $p=0.047$); average TBF at day 3 was significantly longer in the EBF group than in the nEBF group (150.8+83.8 minutes vs117.2+97.8 minutes, $p=0.04$). There were no significant differences in average SBF between two groups. For EBF infants, the peaks of FBF, SBF and TBF arose at day 3; FBF decreased to less than 8 times per day from day 120; SBF decreased from 18.6+10.1 minutes at day 3, to 13.6+8.11 minutes at day 180;

TBF decreased from 150.8+83.8 minutes at day 3, to 94.3+61.1 minutes at day 180. For nEBF infants, FBF at each time point was less than 8 times; FBF and SBF arose at day 10, then reduced. For linear regressions, EBF group was significantly higher in FBF at day 3 and 120 (day 3: B=0.26, p=0.01; day 120: B=0.19, p=0.04), and significantly higher in TBF at day 3 (B=0.20, p=0.03).

Infants growth in EBF and nEBF groups

As shown in Figure 3, for t-tests, average LAZ at day 180 was greater in the nEBF than in the EBF group (0.74+1.05 vs 0.33+1.28, p=0.05). LAZ in the two groups were all above zero except for nEBF infants at day 10 (-0.06+1.18). The curve of average WAZ in the EBF group located above the curve in the nEBF group, but there were no significant differences between the two groups. Average WLZ were significantly gtreater in the EBF group than in the nEBF group at day 120 and 180 (day 120: 0.88+1.08 vs 0.36+1.1, p=0.01; day 180: 1.1+0.94 vs 0.54+1.07, p<0.01). For linear regressions, for WAZ there were no significant differences between EBF group and nEBF group; LAZ was significantly gtreater in the nEBF group than in the EBF group at day 180 (B=-0.21, p=0.02); WLZ were significantly gtreater in the EBF group than in the nEBF group at day 120 and 180 (day 120: B=0.24, p=0.01; day 180: B=0.27, p<0.01).

Macronutrient contents of HM in EBF and nEBF groups

As shown in Figure 4, for t-tests, the average protein content in the HM in the nEBF group at day 10 was significantly higher than in the EBF group (1.23+0.09 g/mL vs 1.18+0.11 g/mL, p=0.01); the average lactose contents in HM in the nEBF group at day 10 and 60 were significantly higher than in the EBF group (day 10: 7.65+0.56 g/mL vs 7.33+0.69 g/mL, p<0.01; day 60: 7.23+0.4 g/mL vs 7.07+0.42 g/mL, p=0.03). There were no significant differences in the average fat and energy contents between the two groups. For linear regressions, average protein and lactose contents in HM were significantly higher in the nEBF group at day 10 (protein: B=-0.20, p=0.04; lactose: B=-0.21, p=0.04).

DISCUSSION

This study was planned to recruit EBF infants, but the implementation of EBF was compromised by many difficulties in practice. Less than half of the 130 mothers exclusively breastfed their infants for 0-4 month. During the follow-up, there were two challenging periods to implement EBF for 6 months: the first period was the initial stage of breastfeeding, in which a lot of mothers added formula for their infants, due to lack of timely and sufficient breastfeeding guide services; the second period was the complementary feeding stage, in which the mothers preferred to introduce complementary food for their infants before 6 month, due to various reasons, including short maternity leave which made it difficult to continue exclusively breastfeeding.[14,15] In this study, the frequency of breastfeeding per day in EBF infants was higher than that in nEBF infants; the average sucking time per breastfeeding session in the two groups was similar; the total breastfeeding time per day in the early birth days for EBF infants was longer than for nEBF infants. Thus, in nEBF infants the intake of formula replaced the intake of HM, due to the lack of breastfeeding frequency. Therefore, it's crucial for government to provide enough supporting service for breastfeeding to strengthen nutrition counselling during antenatal and postnatal sessions and to extend maternity leave to promote EBF for 6 months.[16]

The average weight of EBF infants was not less than that of nEBF infants, and the length of EBF infants in the first 2 months was not shorter than that of nEBF infants. This were differs t from some previous studies. Infants in developed countries who follow WHO feeding recommendations showed a deceleration in weight and length gain.[12] Also, A study from Japan did not find the difference in weight and length of 1month infants between EBF and other feeding practices.[17] A study in rural Gambia suggested that EBF had limited benefit to infant growth.[18] But in a study from developing countries such as Malawi, the advantage of EBF in promoting infant weight and length was obvious.[19] Differences in those studies might be caused by sample size and different time points of study. In our study, during the time of introduction of complementary food, the average length of nEBF infants was greater. This shows that, during the time of introduction of complementary food, EBF infants tended to have a deceleration in length gain, which may due to iron deficiency anaemia.[20] However, problems co-existent with EBF need not be attributed to it and may be solved in their own right. Ways to optimise breast feeding within a food culture using criteria for infant growth, such as those provided by WHO for EBF infants, are more likely to inspire maternal breastfeeding confidence and later child health than is the quest for greater linear growth with inappropriate complementary feeding. Growth velocity is a double-edged sword, and it is not absolutely the greater the better. From the results of epidemiological studies, the link between growth velocity and cancer, especially breast cancer, is also a problem.[21]

The average protein and lactose HM contents were higher for nEBF mothers than EBF mothers in the early days after birth. Preterm infants from India received HM higher in triglyceride in nEBF mothers than in EBF mothers, with no differences in protein and lactose contents between the two groups of mothers.[22] Previous studies reported that some factors had effect on protein and lactose contents in HM. Some studies found that mothers suffering diet-controlled gestational diabetes had lower protein in HM.[23] Some studies found that lactoferrin which was a bioactive protein in HM was higher in infected mothers, and was higher in mothers of ill infants.[24] Seldom study reported factors that influenced HM lactose contents and no studies found significant relations between HM lactose and maternal diets.[25] It's supposed that lack of EBF caused milk stasis, then might provide a survival advantage to the infants.[22] The findings in our study suggested that different breastfeeding practices in the early birth days, indirectly had relationship with breast milk composition. More studies are needed to explore how feeding practices have relations with macronutrients contents in HM.

Conclusions

For nEBF infants, intake of formula replaced intake of breastmilk, related to low breastfeeding frequency, which didn't bring weight gain for nEBF infants. The introduction of complementary food inEBF infants support encouraged greater linear growth and WLZ. The present study does not allow any conclusion about the longer-term health implications for better or worse with this association. There may well be environmental, cultural, and intergenerational factors in play here as happens with birth weight and stunting as opposed to healthy shortness.[26,27] Different breastfeeding practices indirectly had relationship with HM macronutrients contents. Therefore, lactation mothers need help to take up challenges, including appropriate complementary feeding and maternal leave extension.

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AUTHOR DISCLOSURE

The authors have declared that no conflicting interests exist.

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Table 1. Frequency Distributions of Participants (N=130)

	Total n (%)	EBF n=59 n (%)	nEBF n=71 n (%)	χ^2	*p*
Regional distribution					
The northern China	65 (49.2)	23 (39.0)	41 (57.7)	4.54	0.03
The Southern China	66 (50.8)	36 (61.0)	30 (42.3)		
Gender					
Male	69 (53.1)	30 (50.8)	39 (54.9)	0.22	0.64
Female	61 (46.9)	29 (49.2)	32 (45.1)		
Birth way					
Natural spontaneous delivery	66 (52.8)	31 (56.4)	35 (50.0)	0.50	0.48
Caesarean section	59 (47.2)	24 (43.6)	35 (50.0)		
Maternal education level					
Junior high school and below	23 (18.1)	7 (12.5)	16 (22.5)	9.00	0.03
Senior high school	29 (22.8)	15 (26.8)	14 (19.7)		
Undergraduate	68 (53.5)	34 (60.7)	34 (47.9)		
Postgraduate and above	7 (5.5)	0 (0.0)	7 (9.9)		
Maternal occupation					
Government and institution	17 (13.3)	7 (12.3)	10 (14.1)	5.63	0.13
Enterprise	36 (28.1)	12 (21.1)	24 (33.8)		
Business and services	22 (17.2)	8 (14.0)	14 (19.7)		
Housewife	53 (41.4)	30 (52.6)	23 (32.4)		

Table 2. General Information of Measurement Data for Participants (N=130)

	Total M±SD	EBF n=59 M±SD	nEBF n=71 M±SD	*t*	*p*
Birth length (cm)	50.5±1.70	50.6±1.94	50.5±1.49	0.24	0.81
Birth weight (g)	3389.7±436.6	3417.7±457.0	3367.3±421.4	0.65	0.52
Maternal prenatal BMI (kg/m^2)	30.6±8.44	31.5±9.16	29.8±7.82	1.14	0.26
Maternal age (year)	27.9±3.60	27.5±3.63	28.2±3.57	-1.04	0.30

BMI: body mass index.

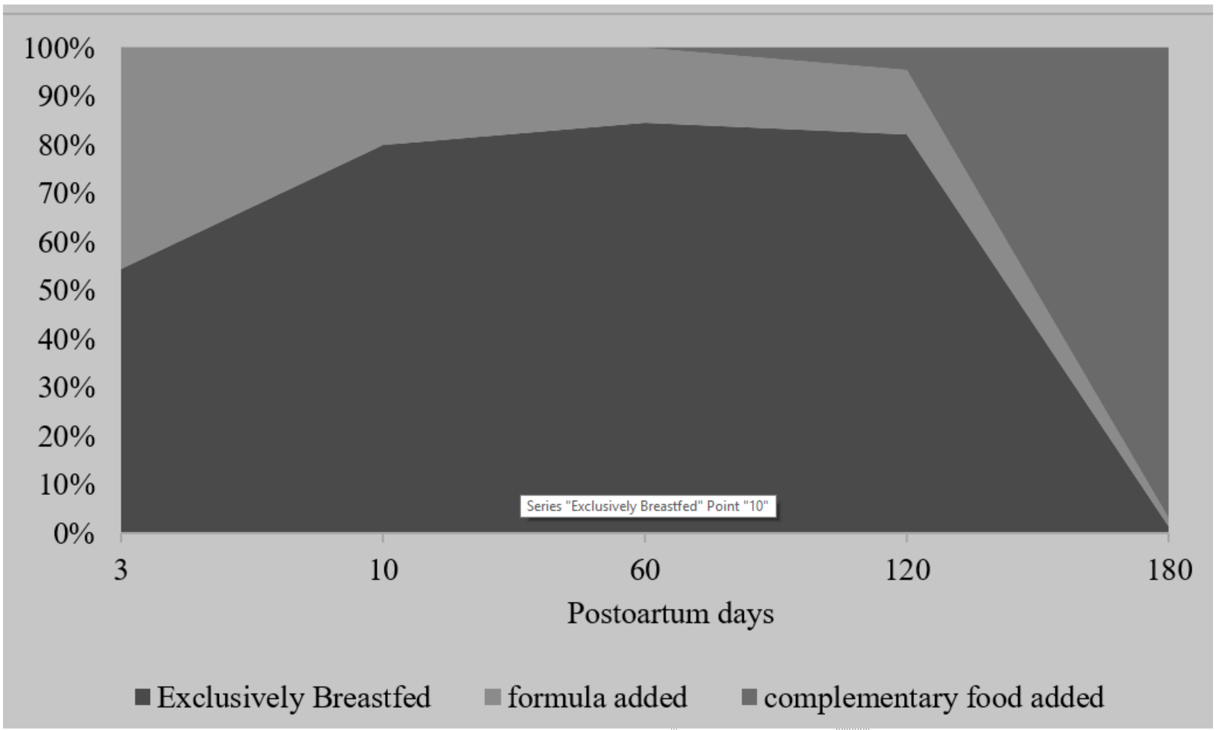

Figure 1. Infant feeding practices by postpartum day.

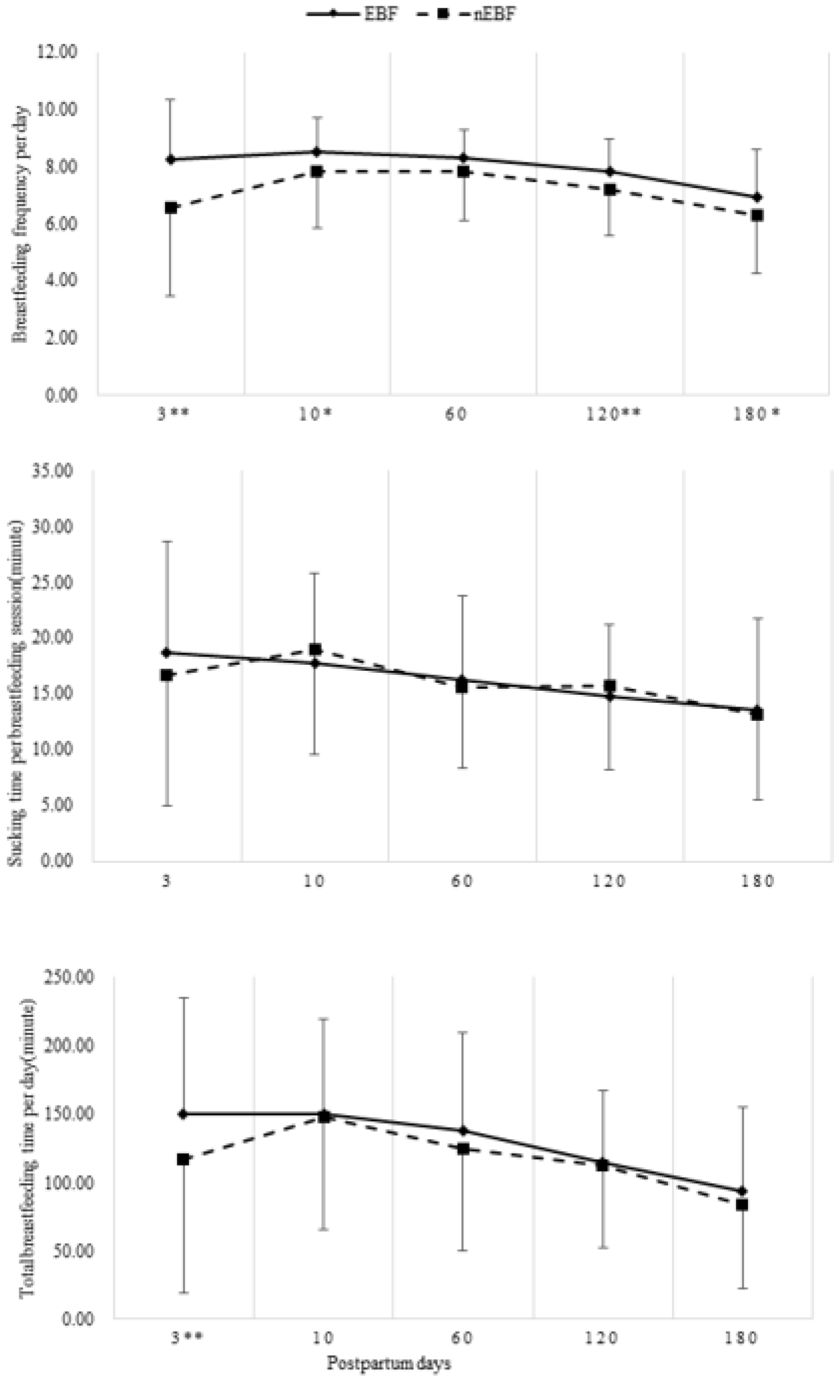

Figure 2 Breastfeeding practices for infants of EBF and nEBF groups.
T-tests and linear regressions were used for comparison.
Values of t-tests are: FBF: day 3: t=3.76, *p*<0.05; day 10: t=2.31, *p*=0.02; day 60: t=1.93, *p*=0.06; day 120: t=2.42, *p*=0.02; day 180: t=2.01, *p*=0.047. SBF: day 3: t=1.02, *p*=0.31; day 10: t=-0.79, *p*=0.43; day 60: t=0.44, *p*=0.66; day 120: t=-0.81, *p*=0.42; day 180: t=0.36, *p*=0.72. TBF: day 3: t=2.08, *p*=0.04; day 10: t=0.16, *p*=0.88; day 60: t=1.03, *p*=0.31; day 120: t=0.23, *p*=0.82; day 180: t=0.98, *p*=0.98.
Standardized slopes in linear regressions of EBF have been adjusted for sex, birth weight, city, birth way, season, maternal age, maternal occupation and maternal education level. Values of standardized slopes are: FBF: day 3: B=0.26, *p*=0.01; day 10: B=0.17, *p*=0.09; day 60: B=0.15, *p*=0.12; day 120: B=0.19, *p*=0.04; day 180: B=0.35, *p*=0.23. SBF: day 3: B=0.13, *p*=0.19; day 10: B=0.03, *p*=0.79; day 60: B=0.05, *p*=0.64; day 120: B=-0.02, *p*=0.87; day 180: B=0.15, *p*=0.09. TBF: day 3: B=0.20, *p*=0.03; day 10: B=0.09, *p*=0.36; day 60: B=0.09, *p*=0.34; day 120: B=0.09, *p*=0.37; day 180: B=0.17, *p*=0.08.
**p*<0.05 for t-tests; ***p*<0.05 for both t-tests and linear regressions.

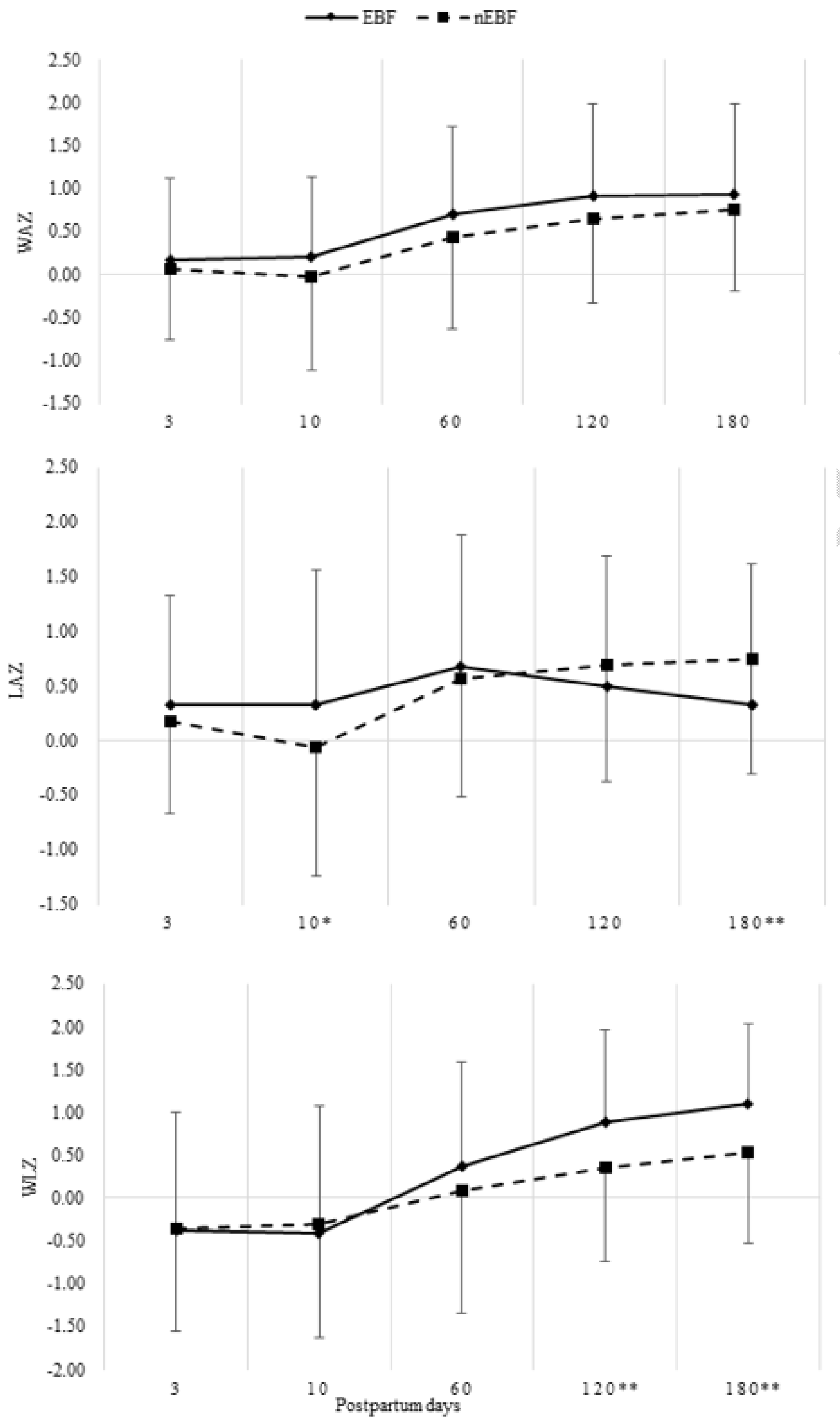

Figure 3 Infants growth for EBF and nEBF groups.
T-tests and linear regressions were used for comparison.
Values of t-tests are: WAZ: day 3: t=0.63, *p*=0.53; day 10: t=1.26, *p*=0.21; day 60: t=1.40, *p*=0.16; day 120: t=1.45, *p*=0.15; day 180: t=1.06, *p*=0.29. LAZ: day 3: t=0.93, *p*=0.35; day 10: t=1.77, *p*=0.08; day 60: t=0.50, *p*=0.62; day 120: t=-0.95, *p*=0.35; day 180: t=-1.98, *p*=0.05. WLZ: day 3: t=-0.12, *p*=0.91; day 10: t=-0.44, *p*=0.66; day 60: t=1.21, *p*=0.23; day 120: t=2.72, *p*=0.01; day 180: t=3.09, *p*<0.05.
Standardized slopes in linear regressions of EBF have been adjusted for birth weight, city, birth way, season, maternal age, maternal occupation and maternal education level. Values of standardized slopes are: WAZ: day 3: B=0.04, *p*=0.48; day 10: B=0.08, *p*=0.18; day 60: B=0.05, *p*=0.57; day 120: B=0.14, *p*=0.12; day 180: B=0.01, *p*=0.89. LAZ: day 3: B=0.09, *p*=0.28; day 10: B=0.12, *p*=0.16; day 60: B=-0.06, *p*=0.52; day 120: B=-0.16, *p*=0.07; day 180: B=-0.21, *p*=0.02. WLZ: day 3: B=-0.02, *p*=0.79; day 10: B=-0.02, *p*=0.83; day 60: B=0.14, *p*=0.14; day 120: B=0.24, *p*=0.01; day 180: B=0.27, *p*<0.05.
*$p<0.05$ for t-tests; **$p<0.05$ for both t-tests and linear regressions.

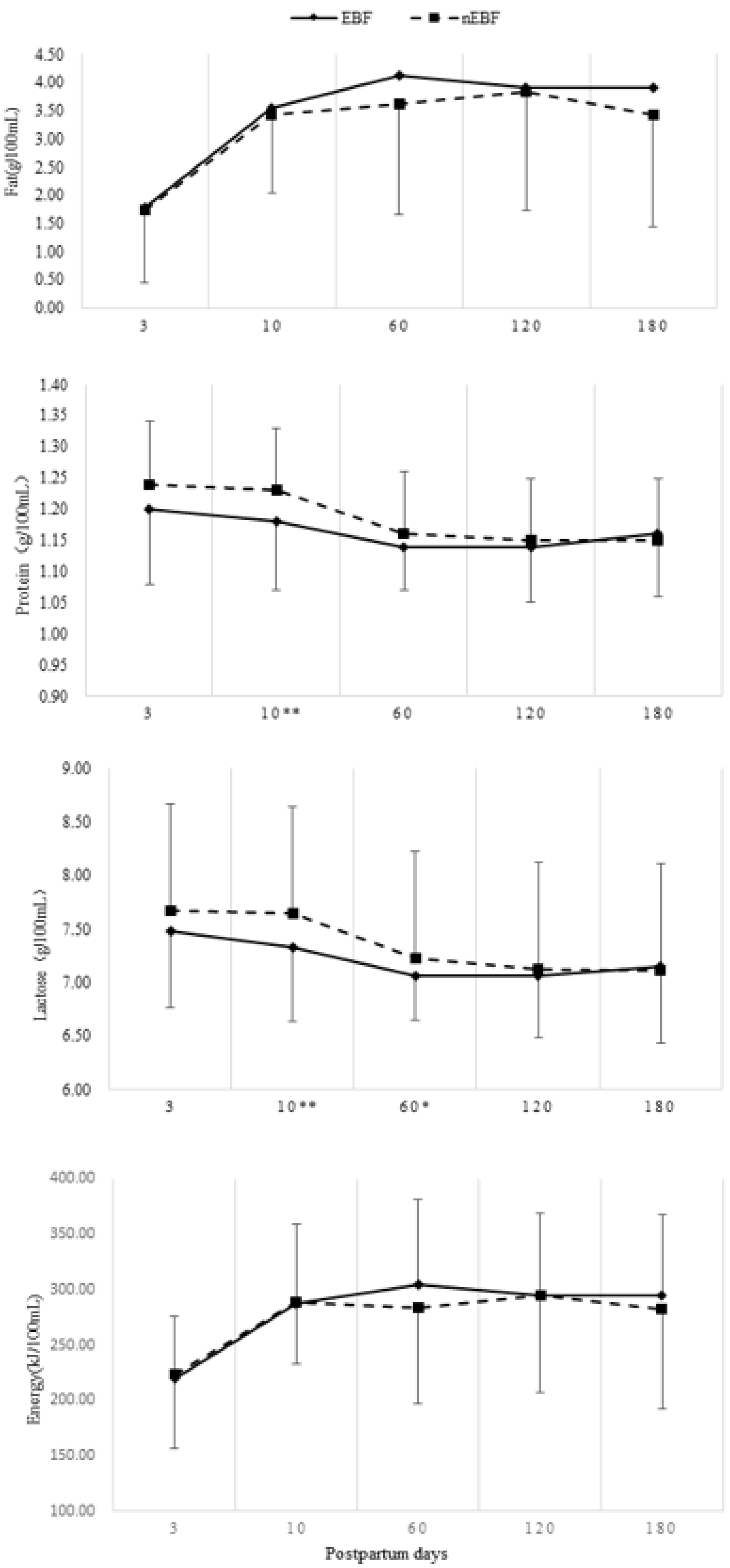

Figure 4. HM macronutrient content of EBF and nEBF group.
T-tests and linear regressions were used for comparison.
Values of t-tests are: Fat: day 3: t=0.24, *p*=0.81; day 10: t=0.46, *p*=0.65; day 60: t=1.44, *p*=0.15; day 120: t=0.15, *p*=0.88; day 180: t=1.26, *p*=0.21. Protein: day 3: t=-1.06, *p*=0.29; day 10: t=-2.89, *p*=0.01; day 60: t=-1.54, *p*=0.13; day 120: t=-0.46, *p*=0.65; day 180: t=0.73, *p*=0.47. Lactose: day 3: t=-1.07, *p*=0.29; day 10: t=-2.91, *p*<0.05; day 60: t=-2.21, *p*=0.03; day 120: t=-0.55, *p*=0.58; day 180:

t=0.35, *p*=0.73. Energy: day 3: t=-0.26, *p*=0.80; day 10: t=-0.12, *p*=0.91; day 60: t=1.32, *p*=0.19; day 120: t=0.04, *p*=0.97; day 180: t=0.71, *p*=0.48.
Standardized slopes in linear regressions of EBF have been adjusted for sex, birth weight, city, birth way, season, maternal age, maternal occupation and maternal education level. Values of standardized slopes are: Fat: day 3: B=-0.03, *p*=0.72; day 10: B=0.001, *p*=0.97; day 60: B=0.08, *p*=0.42; day 120: B=-0.04, *p*=0.69; day 180: B=0.09, *p*=0.32. *P*rotein: day 3: B=-0.11, *p*=0.25; day 10: t=-0.20, *p*=0.04; day 60: B=-0.05, *p*=0.58; day 120: B=0.07, *p*=0.43; day 180: B=0.10, *p*=0.26. Lactose: day 3: B=-0.12, *p*=0.23; day 10: B=-0.21, *p*=0.04; day 60: B=-0.12, *p*=0.22; day 120: B=0.06, *p*=0.53; day 180: B=0.07, *p*=0.47. Energy: day 3: B=-0.08, *p*=0.41; day 10: B=-0.05, *p*=0.64; day 60: t=0.08, *p*=0.41; day 120: B=-0.04, *p*=0.72; day 180: B=0.04, *p*=0.63.
**p*<0.05 for t-tests; ***p*<0.05 for both t-tests and linear regressions.