Effects of relaxation therapy on maternal psychological status and infant growth following late preterm and early term delivery: a randomized controlled trial

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Running tittle

Relaxation effects on maternal and infant outcomes

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Conflicts of interest

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Author Contributions

The authors' responsibilities were as follows: MF, JW, and JY: designed the study; JY and ZW: responsible for data collection and breastmilk composition analysis; JY: conducted the statistical analysis and wrote the first draft under the supervision of MF; MF: led the editing process; and all authors: revised the manuscript and approved the final version. All authors take responsibility for the integrity of the study data.

Trial Registration

This trial was registered at clinicaltrials.gov as NCT03674632.

URL:

https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S00089VA&selectaction=Edit&uid=U00045UL&ts=2&cx=v9xz2g

Abbreviations

BAI Beck Anxiety Inventory

BEBQ Baby Eating Behavior Questionnaire

CG control group

CI confidence interval

ET early term

IG intervention group

IIFAS IOWA Infant Feeding Attitude Scale

LP late preterm

MD mean difference

PSS Perceived Stress Scale

RCT randomized controlled trial

SDS standard deviation score

Abstract

Background: Maternal stress is one modifiable variable that could influence mother-infant signaling and negatively affect breastfeeding and infant growth. This study tested the hypothesis that relaxation therapy would reduce maternal stress and improve infant growth, behavior and breastfeeding outcomes following late preterm (LP) and early term (ET) delivery.

Methods: A single blind randomized controlled trial (RCT) was conducted in healthy Chinese primiparous mother-infant pairs following LP or ET delivery (34^{+0}-37^{+6} gestation weeks). Mothers were randomly assigned to intervention group (IG, listening to relaxation meditation at least once a day) or control group (CG, normal care). Primary outcomes, changes in maternal stress (Perceived Stress Scale (PSS)), anxiety (Beck Anxiety Inventory (BAI)), infant weight and length standard deviation score (SDS), were assessed at 1- and 8-weeks post-partum. Secondary outcomes (breast milk energy and macronutrient composition, maternal breastfeeding attitudes, infant behaviors (3-day diary), and 24-hour milk intake) were assessed at 8-weeks.

Results: 96 mother-infant pairs were recruited. There was a significantly greater reduction in maternal perceived stress (PSS score) in the IG compared to the CG [mean difference (MD)= 2.65, 95% confidence interval (CI) 0.8, 4.5]; and significantly greater infant weight SDS gain (MD= 0.51, 95%CI 0.2, 0.9) from 1-8 weeks. Exploratory analyses showed a significant interaction between intervention and sex, with greater effects on weight gain in female infants. Mothers of female infants used the intervention more frequently with significantly higher milk energy shown at 8-weeks.

Conclusion: The relaxation meditation tape is a simple, effective practical tool that could easily be used in clinical settings to support breastfeeding mothers following LP and ET delivery. The findings need confirmation in larger groups and in other populations

Key words: lactation, breastfeeding, milk intake, maternal stress, infant weight, mother-infant signaling

Introduction

Early life nutrition presents a window of opportunity during which breastfeeding is important for optimizing infant growth, health and development (1-4). However, despite many initiatives breastfeeding rates remain lower than recommended (1).

Mother-infant signaling refers to the way in which infants and mothers communicate and interact (8) and involves maternal physiological and psychological factors, as well as infant physiological and behavioral factors. Lactation is metabolically costly; the daily energy expenditure of lactating women is 25%-30% higher than that of moderately active non-lactating women of average size (9). This is predicted to lead to a 'conflict of interest' between mother and infant over the magnitude of nutritional transfer during breastfeeding, and to a degree of tension in biological and behavioral signaling systems (10). In this context, maternal stress during lactation may impact the physiology of breastfeeding signaling(11). Our previous randomized controlled trial (RCT) (12) investigated mother-infant signaling in 64 healthy term mother-infant pairs and showed that use of a simple relaxation meditation tape during breastfeeding from 2-14 weeks led to significantly reduced maternal stress with better infant growth and longer sleep duration, together with lower hindmilk cortisol and higher carbohydrate consistent with more efficient maternal nutritional investment.

Compared to mothers of term infants, those who deliver early may experience greater stress/increased tension during lactation for reasons including separation when the infant requires intensive care combined with concern about infant wellbeing, and lack of physiological breast stimulation by an immature infant who may initially be too small or sick to breast-feed (7). Several relaxation methods including visual imagery (13), verbal protocols (14), and music therapy (14, 15) have shown beneficial effects on breast milk volume in these mothers, but conclusions are limited by weak study design, high attrition, and/or small sample sizes. Moreover, little research has focused specifically on late preterm (LP, 34^{+0}-36^{+6} weeks) and early term (ET, 37 $^{+0}$-38+6^{+6} weeks) infants who often experience difficulties establishing lactation and are especially susceptible to early breastfeeding failure (16-18), especially in countries such as China where separation is common when infants are admitted to the neonatal intensive care unit for the first 1-3 days after birth (20).

Thus, the aim of this study was to explore the effects of a relaxation intervention on maternal stress and infant outcomes during the early postpartum period in mothers breastfeeding their LP and ET infant, where there is predicted to be greater mother-infant ‘tension’ over resources and therefore potentially greater benefit.

Methods

The detailed methodology for this trial was reported in the protocol (21) and is summarized below. Due to the impact of Covid-19 lockdown measures in Beijing, the 3- and 6-month data collection planned in the published protocol was not completed.

Study design and participants

A single blind parallel RCT was conducted in healthy primiparous mothers who delivered at 34^{+0}-37^{+6} weeks. Eligibility criteria for inclusion in this study were: 1) primiparous, non-smoking mother who aimed to exclusively breastfeed (EBF) the infant for at least two months (infant could receive expressed breastmilk or formula initially but had to be EBF at enrolment); 2) singleton infant born late preterm or early term (34^{+0}-37^{+6}); 3) Mother and infant healthy with no condition expected to affect breastfeeding or infant growth. Eligible mothers were contacted 3-5 days after delivery. After obtaining written informed consent, participants were randomly assigned to either intervention or control group (IG, CG). Data collection was conducted through two home visits around 1-week and 8-weeks postpartum. To ensure consistency of procedures at each study center, all research assistants and nurses involved in the study attended training courses prior to the start of recruitment. Standard operating procedures for the study were printed and posted at each center. The study was approved by the Research Ethics Committee of University College London (ID: 12681/002) and the Department of Child Health, Beijing Children's Hospital (ID: 2018-167).

Randomization, procedures, and intervention

Randomization was stratified by gestational age (34-35 versus 36-37 weeks), delivery method (vaginal versus caesarean) and by the four study centers (Supplemental Figure 1). Subjects were randomly assigned to either IG or CG within each permuted block.

An independent investigator generated the study ID (randomization sequence) using a computer random number generator. Assignments were stored in sealed, opaque envelopes. After confirming eligibility and stratification information, the next relevant envelope was opened by a member of the research team at Beijing Children's Hospital and the allocation to IG or CG provided to the research nurse. Participants were blinded to the randomization until the end of the study; they were aware that the aim of the study was to investigate factors that may make breastfeeding easier for new mothers. Due to the nature of the study, the researchers could not be blinded since additional materials (diary for recording the use of relaxation tape) were collected during the data collection period.

The intervention was a relaxation meditation CD designed for breastfeeding mothers (22). The original CD was transcribed and translated into Chinese language by a certified yoga therapist. The Chinese version of this relaxation meditation was tested and compared with other four relaxation techniques in a pilot study and shown to be the most effective approach for helping breastfeeding mothers to relax (23). Mothers in the IG were given the relaxation meditation recording by scanning a QR code. They were asked to listen to the recording as frequently as possible while breastfeeding or expressing milk, preferably at least once a day. They were also asked to record their use of the tape in a diary. Both groups received standard breastfeeding support and postnatal care, including but not limited to the 1-week home visit and infant feeding advice, monthly telephone interview and free consultation in local clinics based on requirements. There was no difference in the amount of contact with the research team between IG and CG.

Outcomes and measures

Primary outcomes of the study were changes in perceived maternal stress, anxiety, infant weight and length SDS from 1- to 8-weeks. Secondary outcomes reported in this paper are breastmilk macronutrient composition and energy content, infant eating behavior, and maternal feeding attitudes at 8-weeks, 24 hours milk intake at 8-weeks, and infant behaviors (sleeping, awake/happy, and distressed) at 8-weeks.

Baseline characteristics of the participants were obtained using demographic questionnaires. The anthropometry assessment of mother and infants was conducted following standard procedures provided by WHO. Weight and recumbent length of infants were measured using an electronic infant weight and length scale (Betterren-FSG-25-YE, Shanghai, China). Each measure was repeated three times and the mean value used. Infant anthropometric data were converted to standard deviation scores (SDS) based on 21st Intergrowth data (24) to standardize infant weight and length for age and gender. However, given the inclusion of both preterm and early term infants, and the collection of data both before and after term-equivalent, growth analyses were repeated using two alternative approaches: (1) change in SDS based on a combination of 21st intergrowth data (for LP infants) and WHO term infant reference data (for ET infants) (25) and (2) conditional growth based on the standardized residuals for weight and length measurements at 8-weeks regressed on the baseline measurement. Maternal stress, anxiety, feeding attitudes, and infant eating behaviors were assessed using Perceived Stress Scale (PSS), Beck Anxiety Inventory (BAI), Baby eating behavior questionnaire (BEBQ), and IOWA Infant Feeding Attitude Scale (IIFAS) respectively. All questionnaires used in this study are available in Chinese with good validity and reliability (Cronbach's α=0.73-0.95) (26-29). Infant behavior was assessed using a 3-day infant behavior diary. This consists of a “time ruler” for 72 hours, which is divided into 15 minutes segments, and has six categories of behavior: Sleeping; Awake and content; Fussy; Crying; Colic; and Feeding, with a definition of each behavior provided (30, 31). Mothers were asked to shade on the ‘time ruler’ using the appropriate symbol for the infant behavior (supplemental Table 2). The length of shading represented the duration of the behavior. During the data analysis period, a new variable “distress” was generated to describe the total time of “crying” “fussy” and “colic” after removing overlaps; meanwhile, “Awake (happy)” was generated to describe the total time for “feeding”, “awake & happy” and “playing” on the 3-day dairy with any overlaps removed. Data from mothers who provided as least a whole day of behavior records were included in the analysis. The 24-hour milk intake was estimated using the 48-hour test weighing method; the final intake value was obtained after increasing the calculated value by 5% to take into account insensible water losses. (32)

Maternal foremilk samples were collected before a feed at around 10:00 a.m. in the morning during the home visit. Mothers were instructed by a trained nurse on how to express milk using a hand pump (Philips Avent, Netherlands). A total of 20ml foremilk was collected and poured into four sterile specimen jars (5ml per jar).. Samples were frozen immediately in a cooler box with dry ice and then frozen at -80°C in the laboratory of the BCH. For the analyses of macronutrient composition in breastmilk, samples (4-5ml) were thawed at room temperature (27-29°C) and then homogenized using a SX Sonicator (FS-T, SXSONIC, China). The samples were then measured by a near-infrared spectroscopy human milk analyzer (MR-1011, HLIFE, China), after calibration. Formal meetings and seminars were arranged monthly to ensure the study procedures were standardized between centers.

For this study, mothers were defined as EBF if they chose "exclusively breastfeeding" as their current feeding method on the questionnaire and if they also chose "no" as the answer to the question "has your infant ever received any other fluid apart from breast milk?". Milk fortifier for preterm infants and expressed breast milk were included as "EBF" while water and other fluid were not included; details were explained by the nurse who collected the data. Considering that breastfeeding in LP or ET mothers might not be established immediately after delivery; it was acceptable if the baby had received some formula or expressed breastmilk initially. However eligible participants had to be EBF at enrolment.

Sample size calculation

The number of mother-infant pairs required was calculated using the conventional formula (33) for independent sample t-test:

$$N = 16 \times \frac{SD^2}{D^2}$$

(N=number per group, SD=standard deviation, D=Difference between group)

The SD and D were obtained from the results of the MOM study (12), which assessed the effects of relaxation meditation tape on reducing maternal stress assessed by PSS between intervention and control groups (D=3.13, SD=5.00). A sample of 82 mother-infant pairs (41 per randomized group) would allow the detection of a 3.13 points difference in perceived stress measured by PSS between groups at 80% power with a significance level of 0.05. To allow for potential drop-outs or failed measurements, a total sample of 120 infants was planned.

Statistical analysis

Statistical analysis was conducted using SPSS (version 26.0). Frequencies or percentages were presented for nominal or ordinal data. Normality was checked for continuous data by using Q-Q Plots and histograms. For normally distributed data, the mean ± standard deviation (SD) was reported and independent t-test was used for group comparisons; for data with non-normal distribution, the median ± interquartile range was reported and non-parametric analysis (Mann-Whitney or Kruskal-Wallis test) was used for group comparisons. Analyses were carried out for all subjects who had available outcome data according to their randomized group. Differences between IG and CG mothers were compared using independent t-tests for changes in the primary outcomes from 1- to 8-weeks and the values of secondary outcomes (IIFAS, BEBQ, macronutrients composition, energy content, milk intake, and 3-day infant behaviors) at 8-weeks. Pearson correlation was used to examine relationships between variables and Spearman correlation was used to examine dose response effects of the frequency of listening to the relaxation tape on primary and secondary outcomes, with comparisons between mothers of male and female infants. General linear models were used to test for interactions between the intervention and infant gender or gestational age with changes in primary outcomes. $P < 0.05$ was considered to be statistically significant; mean difference (MD) and 95% confidence interval (CI) are presented to show effect sizes.

Results

Recruitment was conducted from October 2018 to October 2020. Figure 1 shows the study flowchart. 96 mothers were randomly assigned to IG (n=48) or CG (n=48). All data were normally distributed, mean and SD were therefore applied and independent t-test was used for group comparisons.

Baseline characteristics of the study population

Table 1 outlines the descriptive characteristics at baseline for IG and CG mothers. No significant differences were found between IG and CG. The mean value of infant birth weight obtained from their clinical record was 2705g in IG (male=2817g, female=2562g) and 2697g in CG (male=2720g, female=2672g). No significant differences in birth weight were found between IG and CG by gender or gestational age group (Supplemental Figure 2 and 3). Mean maternal age was 29.8±3.8 and 29.9±2.9 years in IG and CG respectively. The mean gestational age of infants was 36.1±1.0 weeks in IG and 36.2±0.9 weeks in CG.

Primary outcomes

As shown in Table 2, mothers in the IG had a significantly greater reduction of stress from 1-week to 8-weeks compared to CG mothers. In secondary analyses, baseline maternal stress was not significantly different between groups, while at 8 weeks, IG mothers presented significantly lower stress compared to CG mothers. Maternal anxiety reduced from 1-week to 8-weeks in both IG and CG with no significant difference between groups. In secondary analyses, no significant difference in anxiety was observed between groups at baseline or at 8-week home visits.

IG infants showed significantly greater weight gain from 1-week to 8-weeks compared to control infants (Table 2); while for changes in length, a trend for a decrease in the CG was observed with no change in the IG. Significantly greater weight gain in IG infants was also shown using alternative approaches (Supplemental Table 1).

Secondary outcomes

No significant differences in breast milk energy content, macronutrient composition, maternal breastfeeding attitudes and infant eating behaviors were observed between IG and CG at the 8-week home visit (Supplemental Table 3). IG mothers had a non-significantly greater increase in fat (MD=0.13g/100ml, 95%CI -0.01, 0.26) and energy (MD=1.76 kcal/100ml, 95%CI -0.06, 3.56) from 1 to 8 weeks (Figure 2 and supplemental Table 3).

Both IG and CG mothers had a generally positive perception towards breastfeeding at 8-weeks using the IIFAS with no significant difference between groups. However, IG mothers showed significantly higher disagreement with item 8: “Women should not breast-feed in public places such as restaurants” (MD =0.56, 95%CI 0.07, 1.06) and significantly higher agreement on item 9 “Babies fed breast milk are healthier than babies who are fed formula” (MD=0.58, 95%CI 0.23, 0.94) compared to CG mothers (Supplemental Table 4).

Data from 27 mothers were available for analysis of milk intake (IG=13, CG=14). The mean value for estimated milk intake was 558±42g in CG mothers and 559±36g in IG mothers with no significant difference between groups. A total of 58 returned the 3-day behavior diary whilst 51 of them provided valid data were included in the analysis. As shown in Table 3, no significant differences were found between IG and CG mothers for infant time spent sleeping, awake (happy) or distressed per day. Pearson correlation showed that lower maternal stress at 8-weeks was significantly correlated with longer infant awake (happy) duration per day (r=-0.279, p=0.047) and more frequent awake and happy behavior episodes (r= -0.343, p=0.032). There were no significant differences in baseline socio-demographic characteristics of mothers in IG and CG who provided the milk intake and infant behavior data (data not shown).

Dose-response effects

Overall, 41 of the 48 IG mothers provided a diary recording their use of the intervention. The majority (83%) had listened to the meditation tape for more than 20 days; 71% mothers had listened on more than 28 days, accounting for half of the follow-up period. No significant differences were found in the total duration or average use of the relaxation therapy between mothers of female and male infants, however mothers of female infants reported significantly more days of listening to the therapy compared to mothers of male infants (45±10 vs. 34±16 days, MD 11 days, 95%CI -19.5, -2.2).

Table 4 shows correlations between the use of the relaxation tape (mean/total duration, days of usage) and maternal stress, anxiety, infant weight and length gain, and infant behaviors. Greater use of the relaxation tape was associated with a greater reduction in maternal stress, while a greater number of days of use was associated with greater infant weight gain (assessed using the SDS calculated by WHO Data).. No significant correlations were found for maternal anxiety and infant sleeping, crying, or awake/happy behaviors.

Exploratory analyses

There was no significant interaction between randomization group and infant gender or gestational age on changes in maternal stress. However, the relaxation intervention showed significantly greater effects on weight gain in female than male infants (*p* for interaction=0.032, Figure 3). In female infants, the intervention result in a 0.93 SDS greater increase in weight gain, whilst in male infants, the intervention contributed to 0.17 SDS greater increase in weight gain, which was not significant. No significant interaction effect was found when using conditional weight gain or the absolute weight gain for the same assessment (Figure 4).

To further investigate the interaction between intervention and infant sex on weight gain, breastmilk macronutrient and energy content changes between male and female infants were compared for both IG and CG, adjusted for infant baseline weight and gestational age. While no significant sex differences were observed in the CG, a significantly larger increase in breastmilk energy from 1- to 8-weeks was observed in mothers of female infants compared to male infants in the IG (2.5±4.8 vs. -0.1±3.6 kcal/100ml, MD 2.6, 95%CI -5.11, -0.05), with a non-significant trend for the change in fat (0.53±0.41 g/100ml for females versus 0.32±0.42 g/100ml for males, MD 0.21, 95%CI -0.39, 0.01). Moreover, as shown in the dose-response analysis, compared to mothers of male infants, those of female infants in the IG spent significantly more days listening to the relaxation tape.

Discussion

This trial evaluated the effects of relaxation meditation on maternal and infant outcomes in Chinese mothers breastfeeding a late preterm or early term infant. A significant dose-response reduction in maternal postpartum stress was observed in IG mothers. IG infants showed significantly greater weight gain from 1-to 8-weeks calculated using both intergrowth and WHO references, with a dose-response effect using the WHO standards, and a trend for greater length gain.

No significant differences were observed for secondary outcomes. The non-significant trend for higher breast milk fat and energy in IG mothers may reflect the collection of fore milk rather than hind milk. If the intervention resulted in a more efficient let-down reflex this might be expected to lead to a greater difference in hind milk fat content which is typically two- to threefold that of foremilk (34-36). This could in turn contribute to the observed greater infant growth. Future studies should ideally collect both fore and hind milk and could also measure serum oxytocin to explore intervention effects on the let-down reflex. In contrast to our previous study, which reported significantly longer sleeping duration in IG infant at 6-weeks (12), we found no significant difference in infant behaviors in this study despite longer sleeping and shorter “distress” duration in the IG. However, these analyses had limited power due to poor compliance with the dairies.

Previous studies have reported faster postnatal growth in male infants than females (37, 38) consistent with findings in CG infants in this study. Based on evolutionary theory (39), Trivers and Willard predicted sex-biased investment in mammalian offspring, whereby mothers in good condition maximize fitness through investing more in sons than daughters. At a mechanistic level, mothers may produce sex-biased milk composition or volume. For example, some animal studies reported greater milk volume, and macronutrients in milk produced for male infant rhesus macaques and calves (40, 41) whilst a small cross-sectional study found 25% greater milk energy at 2-5 months in mothers of male infants (42). However, the results of our study contradict the Trivers-Willard theory as the intervention, which experimentally improved maternal condition, apparently increased maternal investment in female infants more than males. Mothers of female infants in the IG spent significantly more days listening to the relaxation tape, hence greater weight gain in their infants might reflect greater exposure to the intervention leading to a more relaxed mother with a more efficient let-down reflex and, in turn, a higher hind milk intake (43, 44). However, it is also possible that sex-specific infant characteristics influenced maternal compliance with the intervention, although there was no significant difference in infant behaviors between males and females. The tendency in China to prefer male over female infants to continue the family name (45, 46) might have resulted in higher stress levels in mothers of female infants, leading to greater engagement with relaxation therapy. Future studies could explore maternal motivation to use relaxation therapies, or standardize the use of the intervention by mothers of male and female infants although this might be difficult in practice.

It is relevant to consider whether the significantly greater weight gain in IG infants is beneficial. Current evidence suggests there could be a trade-off in preterm infants: providing enhanced nutrition to prevent growth faltering results in better cognitive outcomes, but preterm birth per se has been associated with higher cardiovascular risk (47) and this might be worsened by accelerated postnatal weight gain (48). However, studies generally focus on infants born before 32 weeks GA. Infant weight SDS in the present study were around the median, hence the increased weight gain in the IG might not be regarded as “rapid growth” but rather ‘optimal’. Additional outcomes including lean mass and a longer follow-up period are ideally required to confirm if the increased weight gain seen in these LP and ET infants is indeed beneficial.

Strengths of the study include the use of an experimental approach. Addressing research gaps identified from previous studies, we assessed several outcomes from both psychological and physiological perspectives in mother and infant dyads, instead of focusing on one aspect. The sample size was also larger than previous studies and we targeted mothers who may be more stressed following late preterm/early term delivery, which was hypothesized to lead to a greater intervention effect. Stringent pre-study training and frequent validation of instruments and procedures helped to minimize errors and strengthen internal validity. Mothers were blinded to the use of relaxation therapy, being told only that the aim of this study was to investigate maternal breastfeeding outcomes following late preterm/early term delivery. This minimized the chance that mothers in the control group would seek alternative relaxation interventions. Follow-up rates for primary outcomes were good, as was compliance with the intervention, so dose-response effects could be assessed based on the intervention use dairy.

However, several limitations should also be acknowledged. First, it was impossible to blind research nurses to the intervention as some materials differed for IG and CG mothers. Second, results for maternal stress, anxiety, breastfeeding attitudes and infant behaviors could be biased due to the use of self-reported questionnaires. Although mothers were blinded to the randomization, IG mothers might have expected to feel less stressed after using the relaxation tape or that they were expected to report this by the researcher. However, in our pilot study (23), the relaxation tape produced a significant reduction in objective relaxation responses such as heart rate and blood pressure (50). Hence, acute effects of the relaxation therapy on both physiological and psychological maternal outcomes have been shown. Ideally, objective measurements of the effects of relaxation therapy on mother and infant should be included in future studies; for example, some studies have used audio recording for the assessment of infant behaviors (51, 52), although maternal acceptance of such monitoring instruments might be problematic. Finally, we did not formally correct for multiple testing and this should be considered when interpreting the results.

In conclusion, this study showed that a simple relaxation intervention during breastfeeding reduced maternal stress and increased infant weight gain following late preterm or early term delivery. Greater use of the intervention by mothers of female infants may explain the significantly greater weight gain in IG girls and highlights the importance of considering potential effects of infant sex on compliance in future studies. The findings are consistent with the hypothesis that more relaxed mothers may invest more in their infants by producing milk with more favorable nutritional composition. They also have clinical relevance since the relaxation therapy is a simple and non-invasive tool which could be played on a smart phone by mothers who have difficulty establishing breastfeeding in clinical settings or by women in the community, including those following post-partum confinement practices in China and other Asian countries or expressing milk after returning to work.

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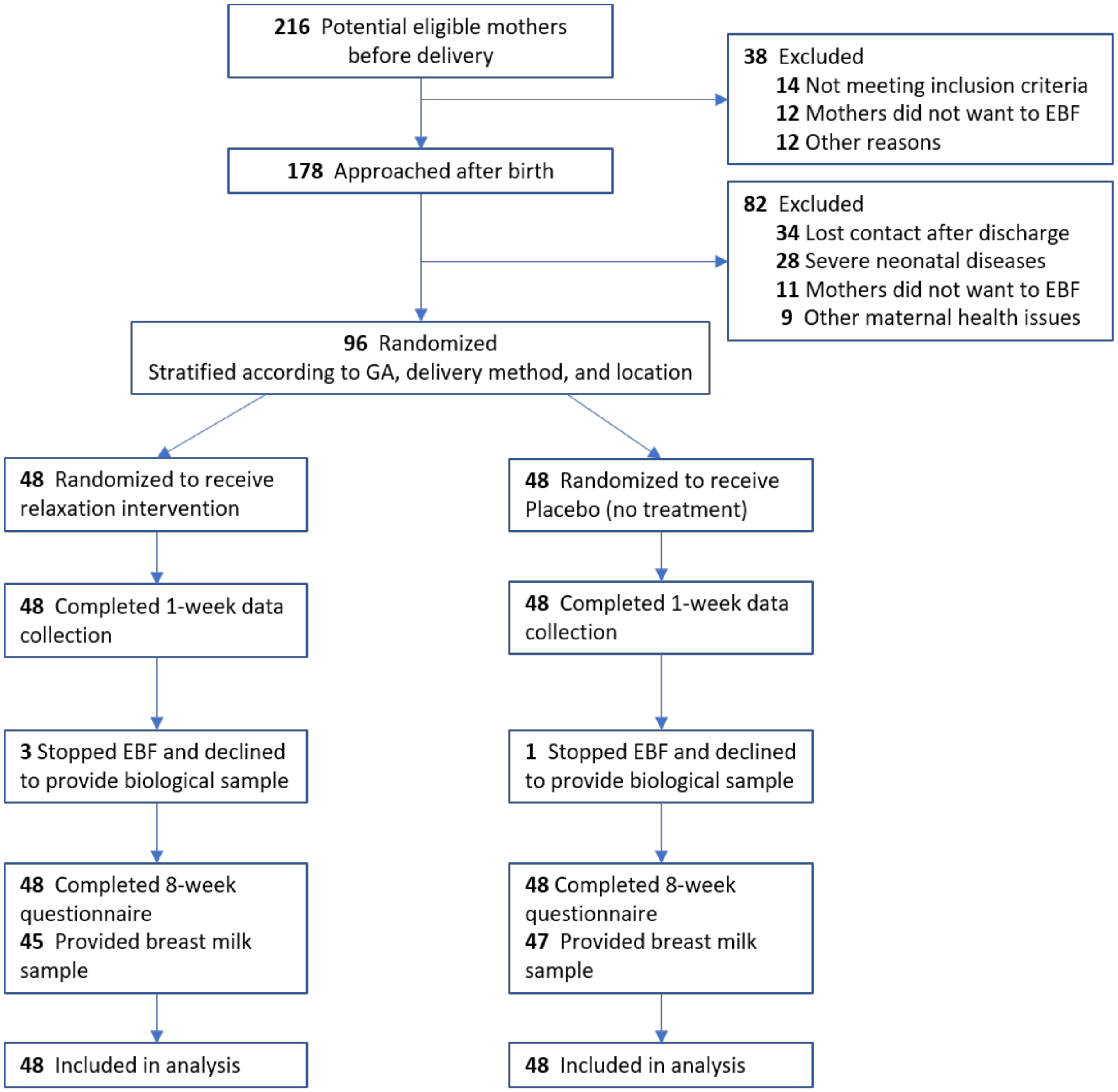

Figure 1. Flow chart of the randomized controlled trial

Notes: The flow chart shows the recruitment process of the randomized controlled trial conducted between October 2018 and October 2020. 96 mothers were randomly assigned to IG (n=48) or CG (n=48). 92 mothers provided breast milk samples. Three mothers (two IG, one CG) declined to provide a breast milk sample; one mother in the IG left Beijing earlier than expected and could not provide all biological samples, but posted her 8-week questionnaires to an investigator. Finally, 48 mother-infant dyads in each group were included in the analysis. GA= gestational age; EBF=exclusive breastfeeding

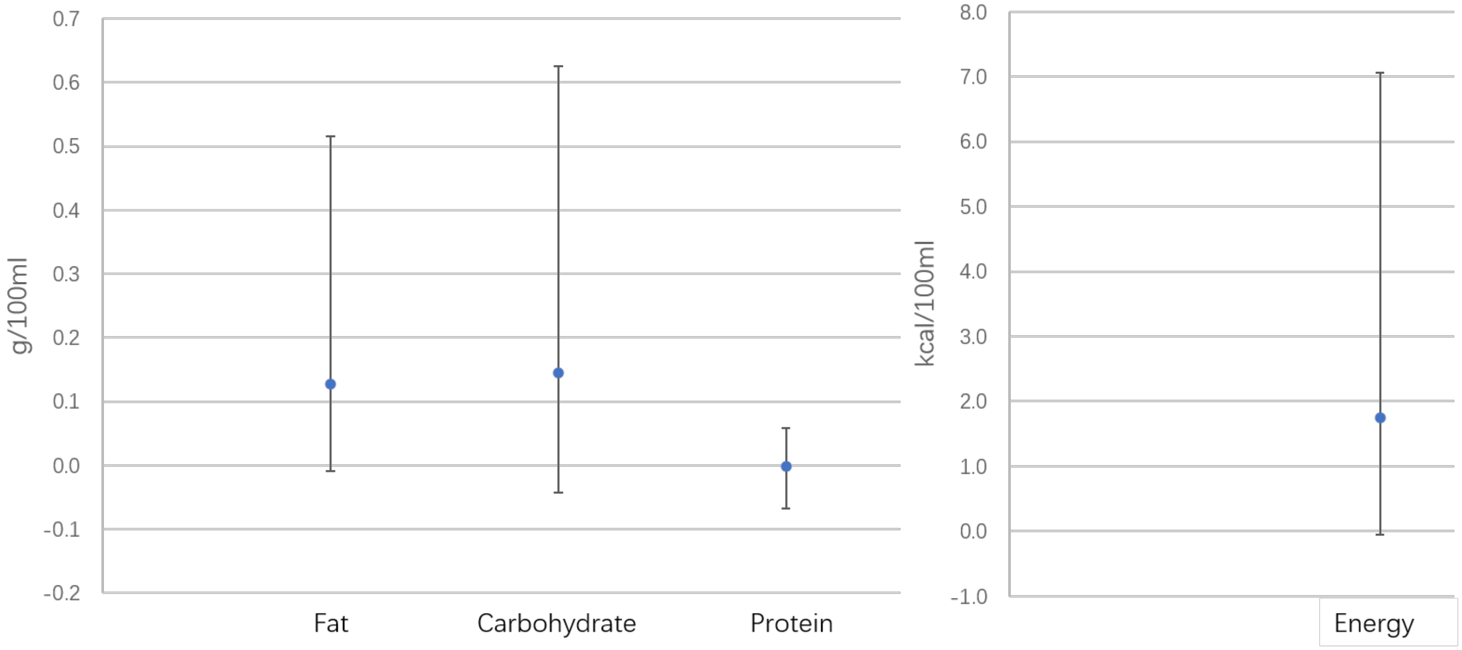

Figure 2. Breast milk macronutrient composition and energy change from 1- to 8- weeks.

Notes: X-axis shows the breastmilk composition (macronutrients and energy content), the Y-axis shows the mean differences of the change in value from 1-8 weeks between intervention and control groups. Error bars shows the 95% confidence interval of the differences. Number of subjects included in the analysis=92, including=45 (female=20, male=25), and CG=47 (female=22, male=25).

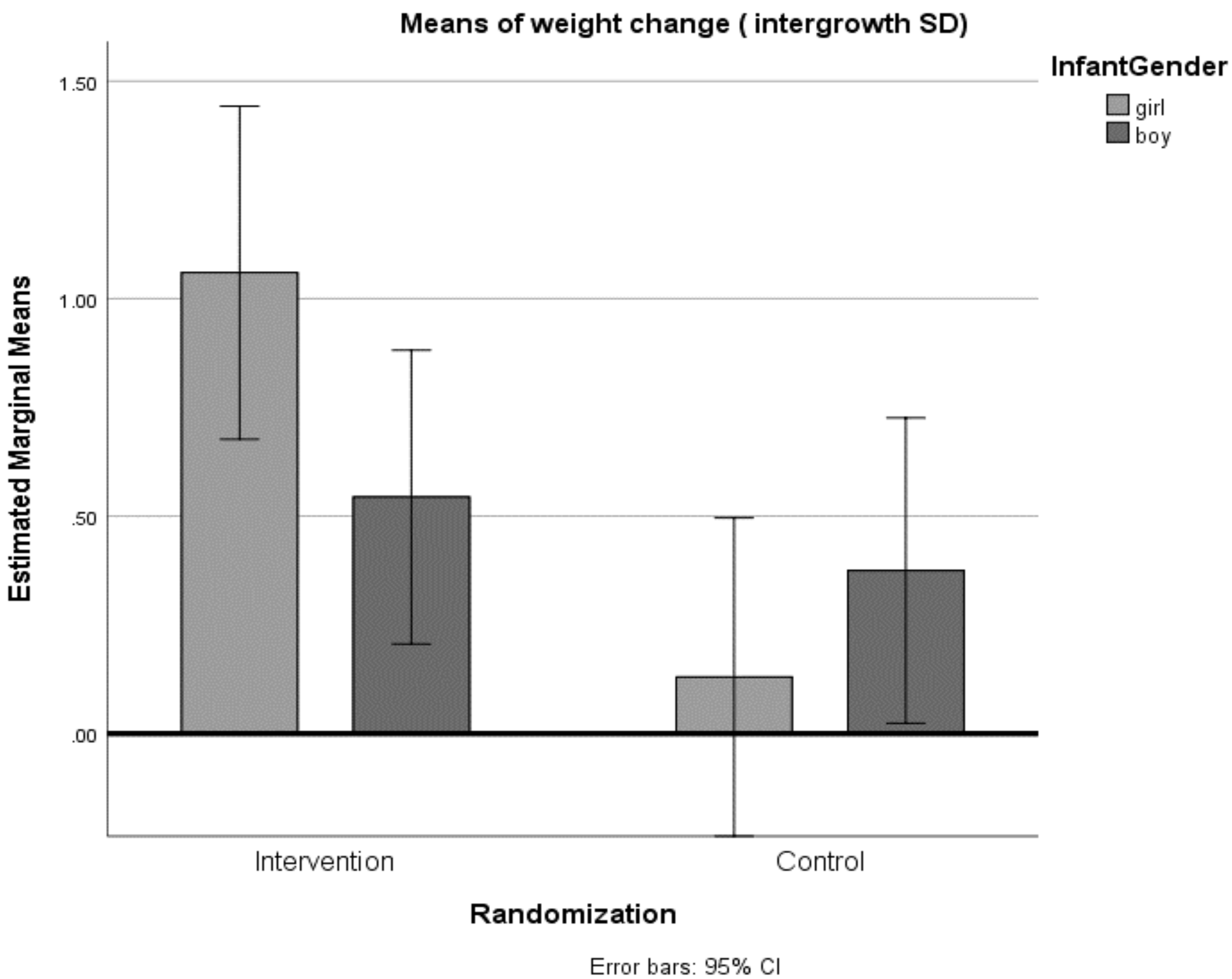

Figure3. Gender differences in weight gain by randomized group using 21st intergrowth SDS.
Notes: Weight gain was measured using standard deviation score (SDS) calculated using the 21st intergrowth data. X-axis shows the gender groups in intervention and control group, the Y-axis shows the mean value of the change in SDS from 1-8 weeks for each group. SD=standard deviation; CI=confidence interval.

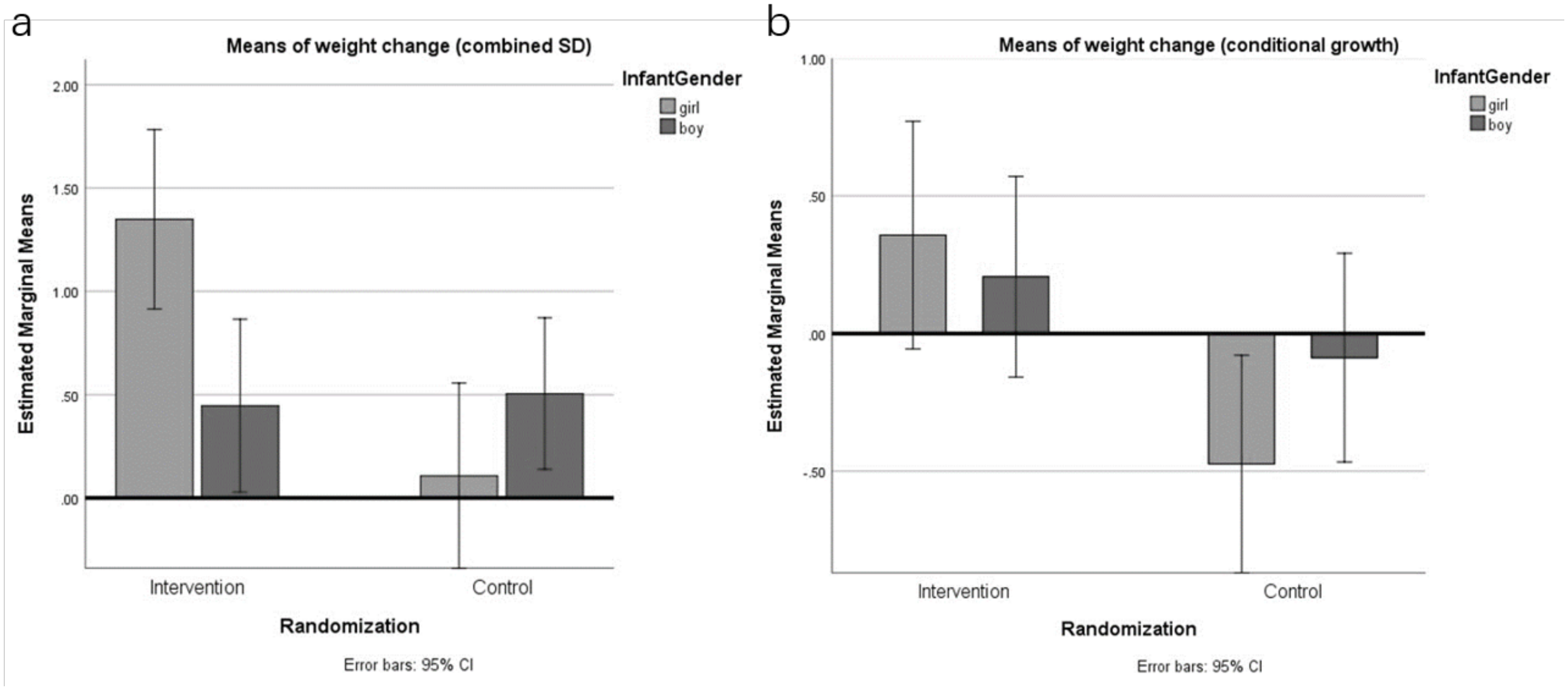

Figure 4. Gender differences in weight gain by randomized group using (a) combined WHO and intergrowth SDS and (b) conditional growth data.

Notes: X-axis shows the gender groups in intervention and control group, the Y-axis shows the mean value of the change in SDS from 1-8 weeks for each group. SD=standard deviation; CI=confidence interval.

a Weight gain was measured using standard deviation score (SDS) calculated using the combined WHO for term infants (37 weeks in the present study) and 21st intergrowth data (for 34-36 weeks infants in the present study).

b Weight gain was measured using conditional growth data. Conditional weight gain was calculated based on the standardized residuals for weight in the study population. The standardized residuals were saved from a regression with the measurements at 8-weeks as the dependent variable, and the measurements at 1-week and time between visits as covariates.

Table 1. Baseline characteristics of the study population.

Descriptive characteristics	Relaxation (n=48)		Control (n=48)		p-value*
	N	%	N	%	
Infant gender					0.84
Male	27	56	25	52	
Female	21	44	23	48	
Gestational age (weeks)					1.00
34 (0/7-6/7)	4	8	3	6	
35 (0/7-6/7)	8	17	7	15	
36 (0/7-6/7)	17	35	18	38	
37 (0/7-6/7)	19	40	20	42	
Location of recruitment					0.98
Northeast Beijing (Centre A)	19	40	20	42	
Central Beijing (Centre B)	6	13	5	10	
Northwest Beijing (Centre C)	11	23	10	21	
South Beijing (Centre D)	12	25	13	27	
Maternal age group (years)					0.72
20-25	5	10	3	6	
26-30	25	54	27	56	
31-35	14	29	15	31	
>35	4	6	3	6	
Marital status					
Married	48	100	48	100	
Educational level					0.75
School	3	6	1	2	
Certificates/Diploma	12	25	11	23	
Bachelor degree	26	54	30	63	
Postgraduate	7	14	6	13	
Household income (CNY/year)					0.44
<200,000	20	41	20	42	
200,000-300,000	13	27	18	38	
300,000-450,000	6	12	6	13	
>450,000	9	18	4	8	
Birth hospital					0.76
Public hospital	43	90	41	85	
Private hospital	5	10	7	15	
Hospital stay after birth					0.58
Less than 48 hours	4	8	6	13	
48-72 hours	14	29	10	21	
More than 72 hours	30	63	32	67	

Main maternity care person					0.77
Husband	32	67	28	58	
Parents	10	21	14	29	
In-laws	5	10	4	8	
Confinement lady	1	2	2	4	

Notes: *Significance examined by using Chi-Square. CNY=Chinese Yuan. 1 GBP=8.32 CNY.. [a] confinement lady: a nanny who is professional in taking care of new mothers.

Table 2. Comparisons of the primary outcomes between randomised groups.

Groups		**Control**	**Relaxation**	**T-test**		
	n	Mean (SD)	Mean (SD)	*p*	MD	95%CI
Stress [a]						
1-week	96	20.19 (6.8)	20.08 (8.1)	0.94	-0.10	-3.1, 2.9
8-week	96	19.92 (5.9)	17.17 (6.6)	**0.035**	-2.75	-5.3, -0.2
Δ	96	0.27 (5.0)	2.92(4.2)	**0.006**	-2.65	-4.5, -0.8
Anxiety [b]						
1-week	96	8.5 (5.8)	8.4 (6.4)	0.95	-0.08	-2.5, 2.4
8-week	96	6.9 (4.8)	5.5 (5.2)	0.18	-1.40	-3.4, 0.6
Δ	96	1.5 (4.3)	2.9 (3.9)	0.12	-1.31	-3.0, 0.4
Weight gain [c]						
1-week	96	0.76(0.8)	0.71(0.7)	0.73	0.05	-0.3, 0.4
8-week	96	1.01 (0.9)	1.48(0.8)	**0.011**	-0.46	-0.8, -0.1
Δ	96	0.26(0.9)	0.77(0.9)	**0.006**	-0.51	-0.9, -0.2
Length gain [c]						
1-week	96	0.88(0.9)	0.93(0.7)	0.73	-0.06	-0.4, 0.3
8-week	96	0.39(1.1)	0.72(1.0)	0.13	-0.33	-0.8, 0.1
Δ	96	-0.37(1.1)	0.01(1.0)	0.08	-0.38	-0.8, 0.1

Notes: [a] assessed by Perceived stress scale (PSS), higher values mean higher stress; [b] assessed by Beck anxiety inventory (BAI), higher values mean higher anxiety; **Δ:** the absolute value of the 8-week value minus the 1-week value; [c] changes of SDS calculated using the 21st intergrowth data for both preterm and term infants; SD=standard deviation; MD=mean difference; CI=confidence interval.

Table 3. Total duration (minutes) of infant behaviour during a day at 8-weeks by randomised group.

	Control			**Relaxation**			**T-test**			
Variables	n	Mean	SD	n	Mean	SD	Sig	MD	95% CI	
Sleeping	25	794	150	26	827	141	0.43	-32.7	-114.5	49.2
Awake (happy)	25	340	127	26	359	114	0.57	-19.4	-87.2	48.5
Distress	25	66	62	26	55	64	0.53	11.0	-24.2	46.3
Distress (record)*	20	82	58	21	68	64	0.46	14.5	-24.3	53.2

Notes: MD=mean difference. *Distress (record) = results including mothers who reported at least one of the "crying" "fussing" and "colic" behaviors while Distress = results including all 51 mothers and assuming the value was 0 if the mother did not provide any record of "crying" "fussing" and "colic" behaviors.

Table 4. Correlations between use of the relaxation intervention and infant outcomes.

		Total duration (minutes)		Total usage (days)		Duration/day (minutes)	
	N	*r*	*p*	*r*	*p*	*r*	*p*
Maternal Stress							
At 8-weeks	41	0.07	0.650	0.15	0.339	-0.209	0.190
Δ	41	**-0.55**	**0.000**	**-0.58**	**0.000**	**-0.435**	**0.005**
Maternal Anxiety							
At 8-weeks	41	0.16	0.334	0.24	0.125	-0.068	0.673
Δ	41	-0.02	0.913	0.02	0.894	-0.280	0.076
Infant Weight A							
At 8 weeks	41	0.24	0.124	0.25	0.115	0.175	0.274
Δ	41	0.13	0.437	0.06	0.735	0.250	0.115
Infant Weight B							
At 8 weeks	41	0.29	0.065	0.32	0.044	0.196	0.219
Δ	41	0.11	0.505	0.03	0.855	0.233	0.143
Infant Length A							
At 8 weeks	41	0.15	0.360	0.19	0.245	0.021	0.898
Δ	41	0.06	0.702	0.03	0.857	0.112	0.485
Infant Length B							
At 8 weeks	41	0.20	0.214	0.25	0.121	0.072	0.655
Δ	41	0.22	0.175	0.25	0.122	0.001	0.996
Infant Behavior							
Sleeping Duration	22	0.06	0.783	-0.07	0.759	0.385	0.077
Crying Duration	11	-0.35	0.291	-0.16	0.637	-0.353	0.287
Awake/happy Duration	15	-0.08	0.789	-0.11	0.695	-0.195	0.487

Notes: Pearson correlation was used. **Δ:** value of the 8-week value minus the 1-week value; A: SDS calculated using 21st intergrowth data; B: SDS calculated using Combined Data.