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6) **Short running head**: Relaxation intervention on mother-infant outcomes

7) **Abbreviations** list:

BF: Breastfeeding

BMI: Body Mass Index

CI: Confidence Interval

CG: Control Group

RG: Relaxation Group

HV: Home Visit

SD: Standard Deviation

TBW: Total body water

8) **Clinical Trial Registry**: ClinicalTrials.gov (ID: NCT01971216)

Randomized controlled trial investigating the effects of a breastfeeding relaxation intervention on maternal psychological state, breast milk outcomes and infant behavior and growth

Abstract

Background: Biological signalling and communication between mothers and infants during breastfeeding may shape infant behavior and feeding. This signalling is complex and little explored in humans, although it is potentially relevant for initiatives to improve breastfeeding rates. **Objectives:** To investigate physiological and psychological aspects of mother-infant signalling during breastfeeding experimentally, testing effects of a relaxation intervention on maternal psychological state, breast milk intake, milk cortisol levels and infant behavior and growth. **Design:** Primiparous breastfeeding mothers and full-term infants were randomized to relaxation therapy (intervention relaxation group; *n*=33 (RG) or control group (*n*=31 (CG); no relaxation therapy) at two weeks post-partum. Both groups received standard breastfeeding support. Home visits were conducted at 2 (HV1), 6 (HV2), 12 (HV3) and 14 (HV4) weeks to measure maternal stress and anxiety, breast milk intake and milk cortisol, and infant behavior and growth. **Results:** RG mothers had lower stress scores post-intervention than CG (HV3 Δ= -3.13, CI: -5.9, -0.3) and lower hindmilk cortisol at HV1 (Δ=-44.5, CI: -76.1 %, -12.9 %) but not HV2. RG infants had longer sleep duration (Δ=82 mins/day, CI: 16, 149) at HV2 and higher weight and BMI SDS gain than CG (Δ=0.76, CI: 0.3, 1.22; and Δ=0.59, CI: 0.09, 1.1 respectively). RG infants had a mean milk intake at HV3 that was 227 g/day higher than the CG infants (p=0.031) after controlling for gender and milk intake at HV1. **Conclusion:** The trial shows the effectiveness of a simple relaxation intervention for improving maternal and infant outcomes and identifies some potential signalling mechanisms for investigation in future and larger studies, especially in settings where mothers are more stressed such as those with preterm or low birth weight infants.

Keywords: lactation, milk intake, milk cortisol, maternal stress, infant weight, parent-offspring signalling.

INTRODUCTION

Early infancy is a critical period of development and growth during which nutrition has an important impact on long-term health and development (1). Breastfeeding is the gold standard for infant nutrition, and confers short- and long-term health benefits for both infant and mother (2, 3). It has been estimated that increasing breastfeeding rates worldwide to at least 50% could save the lives of more than 800,000 young children, and prevent over 20,000 maternal deaths from breast cancer annually (3), as well as reducing socioeconomic inequalities. However, it is widely recognized that global breastfeeding rates are disappointingly low, with less than half of the world's population exclusively breast-fed during the first five months (3).

Initiatives to improve breastfeeding rates have focussed mainly on providing additional support. Biological and psychosocial aspects have been less explored, although breastfeeding is a dynamic process that involves complex signalling and behavioral negotiation between the mother and the infant (4, 5). For example, early behavior or temperament of breastfed infants has been associated with higher maternal breast-milk and salivary cortisol levels (6-8), whilst no such association was found in formula-fed infants (8), suggesting that mothers may shape infant behavior by the transmission of bioactive factors in milk. Maternal plasma cortisol has been associated with psychological distress during the postpartum period (9, 10), and also in turn with milk yield or production (9). Moreover, significant positive correlations have been reported between maternal plasma cortisol and breast milk cortisol suggesting that cortisol is transferred from maternal plasma to milk (11, 12). Conversely, infant crying and vocalization has been associated with maternal depression (13). These mother-infant factors are clearly inter-related, so it is difficult to define cause and effect using an observational study design (14). Furthermore, the measured milk components are influenced by different breast milk sampling strategies, including time of day, stage of lactation and the use of foremilk, hindmilk or mixed fore/hindmilk samples (15).

A recent systematic review reported that relaxation therapy during breastfeeding could benefit mothers of preterm infants by reducing maternal stress or increasing breast milk volume (16). However, there was no

reported evidence on the effects of maternal traits (e.g. psychological state, breast milk yield) among breastfeeding mothers on infant outcomes such as growth or behavior in early life.

The aim of this study was to investigate physiological and psychological aspects of mother-infant signalling during breastfeeding using a more robust experimental design. We aimed to reduce maternal distress by promoting relaxation during breastfeeding using relaxation therapy in a randomized controlled trial. The trial aimed to improve understanding of maternal-infant factors which influence the success of breastfeeding and to identify modifiable factors which could be used for future interventions to improve breastfeeding rates or duration.

METHODS

Study design and participants

This randomized controlled trial (MOM Study) tested the hypothesis that mothers who listened to relaxation therapy would become more relaxed/less stressed and that this would favourably affect breast milk intake and/or alter breast milk composition, including milk cortisol, with beneficial effects on infant behavior and growth. Details of study design, materials and methods are described in the published study protocol (17). Briefly, healthy first-time mothers (free from serious illness, not on medication, and non-smokers) were recruited during their third trimester from antenatal clinics in Klang-Valley, Malaysia between March and December 2014. Those who delivered a healthy full-term infant with birth weight >2.5kg and were exclusively breastfeeding were included in the study and were randomized into relaxation therapy (intervention; n=33 (RG)) or control group (n=31 (CG)) prior to the first home visit (HV1). After randomization, all mothers and infants were followed up until age 14-18 weeks regardless of breastfeeding status. Mothers in the RG were given the relaxation therapy intervention starting at baseline during HV1. Home visits (HV) were conducted at 2 (HV1 (baseline)), 6 (HV2), 12 (HV3) and 14 (HV4) weeks. Mothers gave written informed consent, and the study was approved by the Medical

Research Ethics Committee (MREC), Ministry of Health Malaysia (ID:13-841-16720) and UCL Ethics Committee (ID:4883). The trial was registered with ClinicalTrials.gov (ID: NCT01971216) and the Malaysian National Medical Research Register (NMMR ID: 16720).

Randomization, procedures, and intervention

Randomization was performed prior to HV1. Participants were not informed about the randomization process and CG mothers were not aware of the use of relaxation therapy by the RG to avoid them seeking or using some form of relaxation therapy; they were informed in the trial summary report when the study was completed. A member of the research team in London who was not involved in data collection generated the randomisation assignments using computer blocks of permuted length (2,4,6). Assignments were held in sealed opaque envelopes (17). There was a low possibility of contamination between randomized groups since home visits were performed over a large geographical area and participants did not have contact with each other.

Mothers in the RG were provided with a relaxation therapy audio-recording to listen to while breastfeeding during each HV 1-3 session, and during the subsequent 2 weeks after each HV (18). The relaxation therapy was a modified audio guided imagery protocol designed for breastfeeding mothers (18). After each HV, mothers in the intervention group were asked to listen to the therapy daily whilst breastfeeding or expressing milk for at least two weeks. They were also encouraged to listen beyond 2 weeks as frequently as they found useful throughout the trial and to record in a diary when it was used. Hence, the duration of the intervention was 12 weeks. Mothers in both groups received standard breastfeeding support during the trial (standard breastfeeding education materials such as pamphlets and a breastfeeding guidance booklet, as well as a list providing contact details of health practitioners in government health clinics, breastfeeding support groups and lactation counsellors in the Klang-Valley area). **Figure 1** shows the timeline and research procedures.

Questionnaires

During enrolment when the participants were in the third trimester of pregnancy (Phase 1 of study), they completed questionnaires on sociodemographic context, and perceptions towards breastfeeding using the Iowa Infant Feeding Attitude Scale (IIFAS). During phase 2 of the study, information about labour and early breastfeeding experience was obtained at baseline (HV1). Mothers were asked to record their infant's behavior in a validated 3-day diary after HV1 and HV2. The amount of time the infant spent sleeping, awake and calm, distressed fussing, crying and colic) was recorded in multiples of 5 minute epochs (19). Mothers also completed validated questionnaires about their psychological state (Perceived Stress Scale (PSS) and Beck Anxiety Inventory (BAI)) after each HV1-3 at their convenience.

Anthropometric measurements

Infant weight, recumbent length and head circumference were measured at each HV using a digital infant weighing machine (brand Seca 834), infant length measuring mat (Rollameter 60, UK) and non-stretchable measuring tape (SECA 212, Germany) respectively as described previously (17). BMI was calculated from the anthropometric data as weight(kg) / length(m^2). Anthropometric data were converted to standard deviation scores (SDS) for weight, height, head circumference and BMI using WHO 2006 growth standard (LMS growth add-in for Microsoft Excel).

Cortisol

During HV1-2, mothers were asked to provide breast milk and saliva samples before and after a breast-feed with (RG) or without (CG) the use of relaxation therapy in order to ascertain the effects of the intervention on cortisol levels within a feed. Samples were stored at -80°C before analysis. Cortisol analysis were performed for samples at HV1 and HV2. Milk and saliva samples (500 µL) were thawed at room temperature for duplicate analyses. Samples were first vortexed and centrifuged at 2500 x g for 20

mins at 4°C and then the fat layer (milk sample only) was removed. The liquid sample was then assayed for cortisol concentration using commercially available ELISA kits (RE52611-IBL International, Germany). The sensitivity limit of this assay is 0.01 μg/dL and the upper range is 3 μg/dL. The intra-assay and inter-assay variation was around 5 and 10% respectively (20).

Stable isotope measurements

Breast milk intake and infant body composition were measured using established isotope dilution methods; specifically, deuterium dose-to-the-mother at HV1 and HV3, and deuterium-dose-to-the-infant at HV4 respectively as described previously (17). Briefly, each mother received orally ~30 g deuterium oxide (^2H_2O) diluted in drinking water. Pre-dose saliva and urine samples (day 0) were obtained from mothers and infants respectively, whereas post-dose samples were collected on days 1, 4 and 14 from mothers and days 1, 3, 4, 13 and 14 from infants. At HV4 (or day-14 post dost from HV3), a second isotope dose (0.05g deuterium/kg body weight) was administered to the infants for infant body composition measurement by calculating total body water (21). Infant urine samples were collected at 5-hour, day 1 and 2 post-infant-dose. Frozen samples were transported to London for analysis using isotope-ratio mass spectrometry (IRMS) (Delta XP; Thermo Fisher Scientific). Total breast milk intake was analysed based on the measurement of $^2H_2O/^1H_2O$ enrichment of the maternal saliva samples and infant urine samples. Calculations of breast milk intake of infants were conducted by fitting the isotopic enrichment (tracer) to a model for milk transfer and water turnover (tracee) from the mother to their babies (22). For infant total body water (TBW), isotope analysis of urine samples provided data to calculate the dilution space (N) using the back-extrapolation method, with the dilution space assumed to overestimate the TBW by a factor of 1.044 (23). Although the majority of participants received the isotope and provided breast milk and infant urine samples, some results were deemed implausible based on the IRMS analysis for infant TBW, possibly because the mothers had fed the infant milk expressed after dosing during the sample collection period, which had not been anticipated. Hence, these data were

excluded from the infant TBW analyses before the randomisation code was known. For breast milk intake calculation, only 30% of the samples were available for analysis as the remainder were unfortunately lost by a third party during storage in the UK.

All biological samples were analysed in duplicate by researchers who were not involved in data collection and were blind to the randomized group.

Primary outcomes

To ascertain the long-term effects of the relaxation therapy intervention, the values at the endpoint were compared between groups for these primary outcomes: milk cortisol and infant behavior at HV2, maternal stress and anxiety, breast milk intake and infant anthropometry at HV3, and infant total body water at HV4. General linear model ANOVA was used to further investigate the effect of the intervention on milk intake at HV3, adjusting for milk intake at baseline and gender and, where appropriate, to explore interactions between the intervention and these variables. Weight and BMI were compared between groups based on the changes between time points (e.g. weight gain from HV1 to 3). To ascertain the short-term or acute effects of the intervention, the changes in breast milk cortisol from foremilk to hindmilk at HV1 were also considered given that the mothers had been exposed to the intervention starting after the measurement of baseline (foremilk, pre-feed) variables at HV1.

Statistics

Sample size was calculated to allow detection of a 0.76 SD difference (24) in milk volume between groups at 80% power with a significance level of α=0.05 (25), based on the effect of relaxation therapy on milk volume of mothers with preterm infants in a previous study (24). 28 mother-infant dyads were

required per group. Allowing for a 10% drop-out rate, we aimed to recruit at least 31 mother-infant dyads per group.

Modified intention-to-treat analyses were performed using univariate analyses (independent t-test and chi-square) to compare the results between groups at individual time points and also the changes between time points. SDS for weight and BMI gains were calculated using the LMS weight or BMI gain function which generates an SD score for gain on the baseline value. Milk cortisol data were transformed to natural logarithms (ln) prior to analysis due to skewed data. The statistical package IBM SPSS (version 23) was used for data analysis with the significance level set at $p<0.05$; p values between 0.05 and 0.1 were regarded as indicating a trend.

RESULTS

244 pregnant women were approached of whom 88 were eligible for phase 1 of the study (Figure 1). A second screening was carried out after birth and 64 mothers were eligible to be randomized into intervention or control groups prior to the first home visit (HV1). Almost all mothers (97%) were followed-up from baseline (HV1) to the final time point of data collection (HV4).

Baseline data prior to intervention (Phase 1)

Socio-demographic data and breastfeeding goals

There were no significant differences between groups for maternal characteristics, infant gender, breastfeeding duration goals or confidence levels for attaining these goals (all $p>0.05$) (**Table 1**). The majority of participants planned to breastfeed for more than 12 months and were confident of achieving their goals (Table 1). Both groups had similar perceptions towards breastfeeding with IIFAS mean scores of 67.6±6.7SD and 66.4±6.3 respectively ($p=0.46$, CI:-1.9, 4.4).

Labour and early postnatal experience

Mothers in both groups received similar maternity support during labour and had similar birth and early breastfeeding experiences, with no significant differences between groups for any variable (all $p>0.05$) as shown in **Supplemental Table 1**. The majority of the mothers had a vaginal delivery (75%), were accompanied by their husband (78%) in the labour room and spent 1-2 nights (72%) in hospital post-delivery. The majority (72%) experienced skin-to-skin contact directly after birth, mostly lasting for less than 20 mins and also were able to breastfeed their infant directly after birth (Supplemental Table 1).

Primary outcomes (Phase 2)

Maternal stress and anxiety

Maternal stress scores (PSS) were not significantly different between groups ($p=0.42$) at baseline (HV1), but RG mothers had a significantly lower stress score at both later time points ($p<0.05$) (**Table 2**). There was no significant difference in anxiety score between groups at later visits (HV2 & HV3).

Breast milk intake (isotope data results)

In small subsamples with data available, both RG and CG showed an increase in breast milk intake between the two home visits (**Table 3**). An average 59% (mean difference = 329 g/day, 95% CI: 119, 539) increase in milk intake was observed in the RG between HV1 and HV3 ($p=0.008$) compared to an average of 39% (mean difference = 208 g/day, 95% CI: 5.6, 410) in the CG ($p=0.045$). Comparing groups, there was no significant difference in the increase in milk intake from HV1 and HV3 between RG and CG (mean difference=121.3 g/day, 95% CI: -155, 397). However, further analysis using GLM ANOVA showed that control group infants had a mean milk intake at HV3 that was 226.5 g/day lower than those in the relaxation group ($p=0.031$) after controlling for gender and milk intake at HV1 (**Table 4**). The intake of male infants was 243.3 g/day lower than female infants after adjusting for groups and

milk intake at HV1 (p=0.028) but milk intake at baseline was not a significant predictor of intake at HV3. The model accounted for 24.3% of the variability in milk intake at HV3 (Table 4).

Maternal cortisol levels

At HV1, there was no significant difference in fore milk cortisol between groups, but RG mothers had significantly lower cortisol concentrations in hindmilk at HV1 than CG mothers, (mean -44.5 s% less (C.I: -76.1 s%, -12.9 s%)). Thus, the RG had a significantly greater reduction (34%) in cortisol concentration within a feed at HV1 than the CG, indicating an acute effect of the intervention. However, there were no significant differences between groups in milk cortisol at HV2, suggesting no long-term effect of the intervention on milk cortisol. The maternal salivary cortisol was not significantly different between groups at HV1 or HV2. (**Table 5**).

Infant behavior (3-day diary)

At baseline (HV1), there were no significant differences between groups (n=46) for the time spent sleeping, feeding, awake or distressed (all p>0.05) (**Table 6**). However, at HV2, RG infants had significantly longer sleep duration than CG, with mean sleep duration of 856±99 versus 774±94 minutes per day in RG and CG infants, respectively. The duration of other individual infant behaviors was not significantly different between groups (all p>0.05). The diary was completed by 78% of subjects (90% RG and 65% CG). There were no significant differences in maternal characteristics or socio-demographic background between those who did and did not complete the diary, within each randomized group (p>0.05).

Infant anthropometry and body composition

Weight, length, head circumference and BMI SDS were not significantly different between groups at birth or HV1. RG infants had significantly higher weight and BMI SDS than CG infants at HV3 (all p-values <0.01, **Table 7**). Weight and BMI gain SDS from HV1 to HV3 were also significantly higher in the RG ($p<0.05$). Length and head circumference SDS were not significantly different between groups at later time-points (all $p>0.05$). In a small subsample with data available, the fat mass (FM) and fat-mass-index (FMI) were not significantly different between groups ($p>0.05$) (Table 6). However, there was a non-significant trend towards higher fat-free-mass (FFM) and fat-free-mass-index (FFMI) in RG infants than those in the CG (FFM: 5.2±0.7 vs 4.7±0.8, p=0.10; and FFMI: 12.9±1.4 vs 11.8±1.7, p=0.09).

DISCUSSION

This trial aimed to fill the research gap identified in a recent systematic review (16), by investigating the effectiveness of a relaxation intervention on both maternal and infant outcomes. The most convincing effects of the intervention were reduced stress levels in mothers and higher weight gain and BMI in their infants. However, the intervention therapy also had significant effects on infant behavior with increased sleeping duration at 6-8 weeks of age, and a greater reduction in milk cortisol concentrations during a feed when the mother was first exposed to the therapy. Taken together, these results suggest that listening to relaxation therapy positively influenced maternal psychological state, making the mother less stressed or more relaxed, with consequent effects on infant behavior and growth, as hypothesised. The effects on infant behavior and growth may have been mediated by changes in milk composition and/or milk intake, although the observed trends in milk intake did not reach statistical significance initially, most likely due to the reduced sample available for this analysis. Consistent with our findings, relaxation therapy was also reported to be effective in two trials conducted in mothers of preterm infants which demonstrated favourable effects of the intervention on breast milk yield (24, 26) and composition (24).

Our trial is, to our knowledge, the first to investigate both psychological and physiological mother-infant factors during breastfeeding in an experimental manner. Psychological mother-infant signalling was apparent, since by experimentally manipulating maternal psychological state we were able to show effects on infant sleep. It is possible that mothers who were less stressed had longer and better quality time to physically bond with their infants (e.g. skin-to-skin or comforting their infant); this could in turn stimulate or facilitate infant sleep. Experimental studies (27, 28), including randomized trials (29, 30) found that kangaroo care (skin-to-skin) promotes better self-regulation of the sleep-wake cycle in infants, characterized by longer quiet sleep duration. It is also possible that more relaxed mothers might sleep longer themselves than controls, and this could also have affected infant sleep duration, given that all mothers and infants in the trial were co-sleeping. An observational study of mothers of preterm infants found that relaxation therapy was associated with a reduction in stress and improvement of maternal sleep quality (31). Experimental studies among adults have

also reported that relaxation techniques, either guide-imaginary recordings (32-34) or music relaxation (34-36), improve sleep quality. Maternal sleep pattern or quality and time spent comforting the infant were not assessed in this trial but would be relevant for consideration in future research. Mothers in the control group had significantly higher anxiety scores at baseline than those in the intervention group but no differences were apparent at later visits. Moreover, further multivariate statistical analysis (not reported here) showed that the trajectory of anxiety scores over time (from HV1-3) did not significantly differ between groups, unlike stress scores which diverged to be significantly different at later visits (HV2-3).

Another explanation for the observed effects of the intervention could be physiological signalling via effects of maternal stress on breast milk composition or/and breast milk volume. Firstly, mothers who were less stressed and more relaxed may have produced milk with altered concentrations of bioactive factors such as cortisol, which may have consequently affected infant behavior. Significant differences in milk cortisol within a feed between groups were only found at the first visit, suggesting the intervention may have been more effective in reducing cortisol concentrations when mothers were first exposed. However, the inconsistent results could also reflect practical issues with the timing of data collection, since several visits at week 6-8 had to be performed in the afternoon due to time or work schedule constraints. Secondly, mothers who were less stressed and more relaxed had more efficient or frequent milk ejection, influencing nutrient intake and hence growth. Using a t-test, non-significant trends were apparent suggesting higher milk intake in intervention group infants at HV3 than those in the control group, consistent with the main study findings. This difference became significant after adjusting for milk intake at HV1 and infant gender in further analysis using ANOVA. However, these results should be regarded as exploratory given the small sample size available for the analysis.

Infants in the intervention group (RG) had significantly higher weight SDS and BMI SDS at 12-14 weeks and also significantly higher gain in weight SDS from baseline to study endpoint. There was no indication that this represented excessive growth. The majority of infants had weight-for-age and BMI-for-age SDS score within ±2 SD throughout the study period and no increment >1 band on the growth chart or >±0.67 SD

between measurements (37) occurred between visits. The mean weight and BMI SDS scores of the intervention group at all visits were also within the expected range according to the WHO Growth standard and slightly below the 50th percentile, showing a close match to the optimal growth of breastfed infants (38). Thus, it is possible that the relaxation intervention allowed the breastfed infants to come closer to the 'ideal' growth pattern. There was a non-significant trend suggesting higher fat-free-mass-index in intervention group infants than those in the control group, consistent with the main study findings.

The main strength of our trial is the use of an experimental design of RCT, which minimizes the potential for confounding. Indeed, no baseline differences were identified between groups in the numerous inter-related factors, including socio-demographic background, social support, prenatal distress and labor experience, which have been reported to contribute to postpartum distress in previous studies (39-41), including a meta-analysis (42). Furthermore, involving only primiparous mothers in the trial reduced variability or potential bias in practices and attitudes towards breastfeeding or caring for a new-born baby.

Our trial also had some limitations. First, no adjustment of sample size or p-value cut-off point was performed for the multiple primary outcomes. Thus, the possibility of a type 1 error should be considered when interpreting the findings. Second, compliance with completion of the 3-day diary was not high, which could be due to the large number of different tasks that mothers were asked to perform over the study period. The completion rate for the diary was higher among RG mothers, possibly because they were also recording the frequency of listening to the relaxation therapy in the log book, or because the infant was sleeping longer. Nevertheless, there were no significant differences in infant behaviors between groups at baseline and/or in maternal characteristics or socio-demographic background between compliant and non-compliant subjects, suggesting that the available data can still be considered representative of the study population. Third, due to the nature of the therapy tool, it was not possible to blind RG mothers or researchers to the intervention. It is possible that the provision of a relaxation tape may have influenced mothers' expectations and, therefore, affected outcomes based on maternal report such as stress and infant behavior. We experienced some issues with the isotope data, particularly the unfortunate loss of samples, which was beyond our control due to the

involvement of a third party during storage, and implausible results from the IRMS analyses (for TBW) showing an increase of isotope levels across time post-dose (isotope levels are expected to decline overtime). The most likely explanation for this is that, during the post-dose sample collection period, these infants were fed with expressed breast milk shortly after mothers received the isotope and which therefore contained high concentrations, resulting in high isotope levels in the samples taken on day 1 and 2 post-infant-dose (or day 14 post-mother dose). In fact, many of the study participants regularly expressed breast milk starting from early lactation, mostly due to the short maternity leave (around 2-3 months) in the country. This was unfortunately not predicted, and had not occurred in our previous studies using the similar protocol, where mothers were not routinely expressing milk (43). Due to these methodological issues, not all results were suitable for inclusion in the analysis, hence resulting in a small sample size and limiting the statistical power to detect differences. The isotope method was chosen since it is non-invasive and does not interfere with the breastfeeding process thus providing a better indication of suckled breast milk. However, a larger sample size and properly following standardised procedure of biological sample collection is recommended for future studies. Fourth, although we were able to demonstrate effects of the intervention on the primary outcomes, the relatively small sample size meant that we were not able to explore the relationships between outcomes, including the order and direction of effects. Finally, the generalisability of our findings may be limited since our study population consisted of primiparous mothers who were Malay and well-educated.

In summary, our trial highlights the importance of minimizing and reducing maternal stress, since the experimental relaxation intervention influenced infant behavior, breast milk cortisol and volume at one time point, and subsequently infant growth. The findings have both scientific and practical relevance; they contribute to current understanding of the physiological and psychological perspective of infant feeding, and also identify aspects that can be addressed to increase breastfeeding success. Given that the intervention tool is simple and practical, it could easily be used in future interventions aimed at increasing the rates and duration of breastfeeding. The fact that the intervention was effective even in healthy mother-infant dyads suggests its use in settings where mothers are more stressed could have a greater impact. It would, therefore,

be worth testing the therapy in clinical settings, for example, in mothers of preterm, low birth weight or growth challenged infants, with a larger sample size trial.

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The contribution of the authors was as follows: MF, JW, and NHMS designed the study; NHMS, FM and MF were involved in developing the intervention tool; NHMS was responsible for data collection; AP and ZJP were responsible for the hormone assays; NHMS and SE were responsible for the mass spectrometric analyzes; NHMS, JW and SE undertook the isotope calculations; NHMS conducted the statistical analysis and wrote the first draft under the supervision of MF; MF led the editing process. All authors revised the manuscript and approved the final version and also take responsibility for the integrity of the study data. The trial sponsor (UCL GOS ICH) had no role in study design, data collection, statistical analysis or data interpretation. All authors declare that they have no conflict of interests.

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TABLE 1

Maternal socio-demographic background, infant gender and maternal breastfeeding plan and goals[1]

Descriptive characteristics:		Groups					Statistical test[1]	
		Control		Relaxation		Total	Chi-square test (X^2)	P-value
		n	%	n	%	n (%)		
Mother's ethnicity	Malay	30	96.8	30	90.9	60 (94)	0.34	0.49
	Others	0	3.2	4	9.1	4 (6)		
Age groups (years)	20-25	10	32.3	11	33.3	21 (33)	5.41	0.08
	26-30	21	67.7	17	51.5	38 (59)		
	31-34	0	0	5	15.2	5 (8)		
Marital status	Married	31	100	33	100	64 (100)		-
Highest educational qualification	School	5	16.1	5	15.2	10 (16)	3.00	0.59
	Diploma	3	9.7	5	15.2	8 (13)		
	Bachelor	21	67.7	18	54.5	39 (61)		
	Postgrad-uate	2	6.5	5	15.2	7 (11)		
Household income (RM)	1500-3000	8	25.8	11	33.3	19 (30)	1.38	0.89
	3001-5000	9	29.0	7	21.2	16 (25)		
	5001-8000	10	32.3	9	27.3	19 (30)		
	8001-10000	2	6.5	4	12.1	6 (9)		
	>10000	2	6.5	2	6.1	4 (6)		
Infant gender	female	20	64.5	19	57.6	39 (61)	0.32	0.62
	male	11	35.5	14	42.4	25 (39)		
Breastfeeding goal (duration in months)	2-6	2	6.5	1	3.0	3 (4.7)	1.96	0.90
	7-12	3	9.7	3	9.1	6 (9.4)		
	13-18	1	3.2	2	6.1	3 (4.7)		
	19-36	25	80.6	27	81.8	52 (79.7)		
Confidence levels based on Likert-scale 1-5: Not (1) to strongly confident (5)	1	6	19.4	2	6.1	8 (12.5)	5.57	0.24
	2	5	16.1	2	6.1	7 (10.9)		
	3	5	16.1	5	15.2	10 (15.6)		
	4	11	35.5	15	45.5	26 (40.6)		
	5	4	12.9	9	27.3	13 (20.3)		

[1]Group comparison was performed using Chi-Square test. RM, Ringgit Malaysia.

TABLE 2

Comparison of maternal stress and anxiety scores between randomized groups[1]

Groups :		**Control**		**Relaxation**			
	n	**Mean (SD)**	**n**	**Mean (SD)**	**p-value**	**Mean diff**	**C.I**
Maternal stress - PSS							
HV1	31	17.28 (5.6)	33	16.27 (4.3)	0.42	-1.01	-3.5, 1.5
HV2	31	16.06 (5.9)	31	12.55 (4.4)	**0.011**	-3.51	-6.2, -0.8
HV3	30	15.10 (6.1)	31	11.97 (4.9)	**0.029**	-3.13	-5.9, -0.3
Maternal anxiety – BAI							
HV1	30	15.23 (8.9)	33	10.48 (71.2)	**0.02**	-4.75	-8.8, -0.71
HV2[2]	31	10.0 (14)	30	6.0 (9)	0.13		
HV3[2]	30	9.0 (12)	31	6.0 (10)	0.24		

[1] All values are mean ± SDs. Group comparison was performed using independent t-test except **[2]**.

[2] Group comparison was performed using Mann-Whitney test, results in median (IQR), p-value >0.05.

PSS, Perceived Stress Score; BAI, Beck Anxiety Inventory.

TABLE 3

Breast milk intake of the intervention and control groups at HV1 and HV3[1]

Groups :	**Control (n=11)**	**Relaxation (n=8)**			
Milk Intake (g/day)[1]	**Mean (SD)**	**Mean (SD)**	**p-value**	**Mean diff**	**C.I**
HV1	534.1 (169)	557.8 (148)	0.756	23.65	-134, 181
HV3	741.8 (184)	886.8 (251)	0.164	144.94	-65.3, 355
Difference HV3-HV1	207.7 (300)	329 (250)	0.366	121.3	-154.5, 397

[1] Group comparison was performed using independent t-test.

TABLE 4

Milk intake (g/day) at HV3 after adjusting for milk intake at baseline (HV1), groups and gender[1]

Variables	B	Standard error	t	p-value	C.I
Intercept	1054	179.2	5.9	<0.001	672, 1436
Control group infants	-227	95.3	-2.4	0.031	-430, -24
Male infants	-243	100.1	-2.43	0.028	-460, -30
Milk intake at HV1	-0.4	0.3	-1.3	0.21	-1.1, 0.3

[1] GLM ANOVA analysis (covariates: randomised groups, infant gender and milk intake at HV1; outcome: milk intake at HV3 (g/day)).

TABLE 5

Comparison of breast milk cortisol (µg/dL) between randomized groups[1]

Groups :		**Control**			**Relaxation**					
	n	**Mean**	**(SD)**	**N**	**Mean**	**(SD)**	**P value**	**Mean different (s%)[2]**	**C.I (s%)[2]**	
Milk Cortisol (µg/dL) at HV1										
Fore	31	0.170	(0.1)	32	0.140	(0.09)	0.22	-19.7	-51.8,	12.3
Hind	31	0.167	(0.1)	32	0.107	(0.07)	**0.007**	-44.5	-76.1,	-12.9
Milk Cortisol (µg/dL) at HV2										
Fore	29	0.116	(0.09)	31	0.152	(0.13)	0.21	26.8	-15.7,	69.2
Hind	30	0.096	(0.07)	31	0.099	(0.06)	0.86	3.2	-33.3,	39.7
Saliva Cortisol (µg/dL) at HV1										
Pre BF	31	0.062	(0.05)	32	0.048	(0.04)	0.21	-26.4	-67.8,	14.9
Post BF	31	0.041	(0.03)	32	0.039	(0.02)	0.72	-6.4	-41.7,	28.9
Saliva Cortisol (µg/dL) at HV2										
Pre BF	30	0.062	(0.04)	31	0.044	(0.04)	0.10	-33.5	-73.7,	6.6
Post BF	29	0.044	(0.03)	31	0.036	(0.03)	0.37	-18.6	-60.1,	22.9

[1]Values are geometric means ± SDs. Group comparison was performed using independent t-test.
[2]Values in sympercent (s%). BF, breastfeeding.

TABLE 6

Duration of infant behaviors (in minutes) based on the 3-day diary record[1]

Infant behaviors	control			relaxation			p-value	Mean different	C.I
	N	Mean	SD	N	Mean	SD			
Sleeping (HV1)									
Post HV1	17	849	120	29	819	133	0.45	-30	-109, 49
Post HV2	14	774	94	23	856	99	**0.02**	82	16, 149
Feeding									
Post HV1	17	268	99	29	234	77	0.21	-34	-86, 19
Post HV2	14	217	96	23	169	58	0.07	-48	-99, 4
Awake and calm									
Post HV1	17	247	101	29	306	112	0.08	59	-8, 125
Post HV2	14	416	112	23	360	107	0.14	-56	-131, 19
Distress (Crying and fussy)									
Post HV1	17	76	65	29	81	69	0.83	5	-37, 46
Post HV2	14	34	55	23	55	77	0.38	21	-27, 69

[1]All values are mean ± SDs. Group comparison was performed using independent t-test.
Post HV1 (at 2 week); Post HV2 (at 6-8 week).

TABLE 7

SDS-scores for infant weight and BMI at baseline and later time-points, and body composition (FM and FFM) at HV4[1]

Groups:	**Control**			**Relaxation**			**T-test**			
	n	**Mean**	**SD**	**n**	**Mean**	**SD**	**p-value**	**Mean diff.**	**C.I**	
Weight SDS										
Home visit 1 (HV1)	31	-0.92	(0.7)	33	-0.56	(0.8)	0.06	0.36	-0.02	0.75
Home visit 3 (HV3)	31	-0.90	(0.8)	32	-0.12	(0.8)	**<0.001**	0.78	0.39	1.18
Weight gain HV1-3	31	-0.32	(0.9)	32	0.44	(1.0)	**0.002**	0.76	0.30	1.22
BMI SDS										
Home visit 1 (HV1)	31	-1.11	(0.8)	33	-0.76	(0.9)	0.10	0.35	-0.07	0.77
Home visit 3 (HV3)	31	-1.48	(0.8)	32	-0.52	(0.9)	**<0.001**	0.96	0.51	1.41
BMI gain HV1-3	31	-0.37	(0.9)	32	0.22	(1.1)	**0.022**	0.59	0.09	1.10
Body composition at 14-18 weeks										
FM (kg)	12	1.05	(0.5)	17	1.4	(0.6)	0.13	0.33	0.10	-0.76
FFM (kg)	12	4.7	(0.8)	17	5.2	(0.7)	0.10	0.47	-0.1	1.0
FMI (kg/m^2)	12	2.6	(1.3)	17	3.5	(1.5)	0.13	0.83	-0.27	1.9
FFMI (kg/m^2)	12	11.8	(1.7)	17	12.9	(1.4)	0.09	1.04	-0.17	2.3

[1]All values are mean ± SDs. Group comparison was performed using independent t-test.
FM, fat mass; FFM, fat-free mass; FMI, fat-mass-index, FFMI, fat-free-mass-index.

FIGURE 1

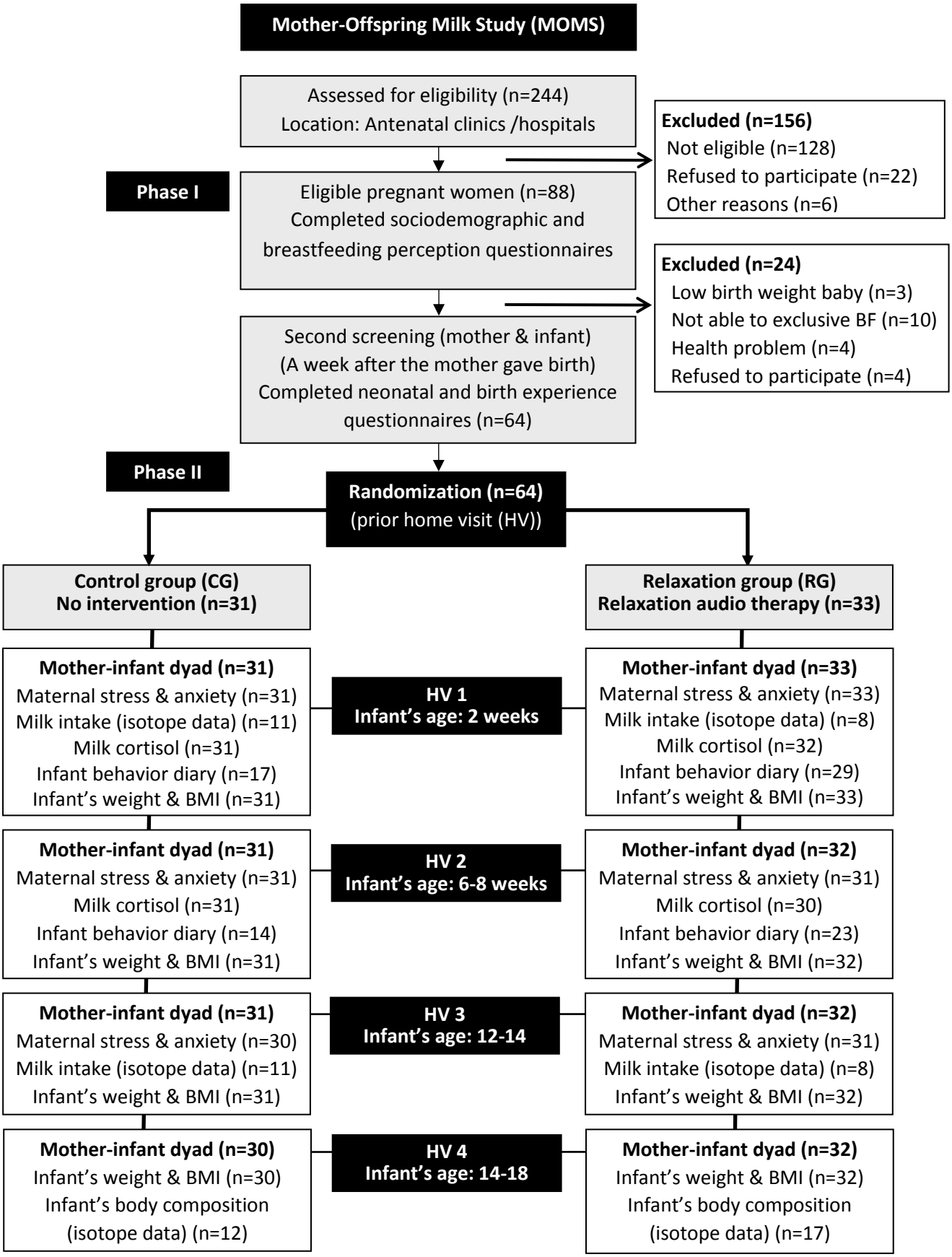
Mother-Offspring Milk Study (MOMS)
Assessed for eligibility (n=244)
Location: Antenatal clinics /hospitals
Excluded (n=156)
Not eligible (n=128)
Refused to participate (n=22)
Other reasons (n=6)
Phase I
Eligible pregnant women (n=88)
Completed sociodemographic and breastfeeding perception questionnaires
Excluded (n=24)
Low birth weight baby (n=3)
Not able to exclusive BF (n=10)
Health problem (n=4)
Refused to participate (n=4)
Second screening (mother & infant)
(A week after the mother gave birth)
Completed neonatal and birth experience questionnaires (n=64)
Phase II
Randomization (n=64)
(prior home visit (HV))
Control group (CG)
No intervention (n=31)
Relaxation group (RG)
Relaxation audio therapy (n=33)
Mother-infant dyad (n=31)
Maternal stress & anxiety (n=31)
Milk intake (isotope data) (n=11)
Milk cortisol (n=31)
Infant behavior diary (n=17)
Infant's weight & BMI (n=31)
HV 1
Infant's age: 2 weeks
Mother-infant dyad (n=33)
Maternal stress & anxiety (n=33)
Milk intake (isotope data) (n=8)
Milk cortisol (n=32)
Infant behavior diary (n=29)
Infant's weight & BMI (n=33)
Mother-infant dyad (n=31)
Maternal stress & anxiety (n=31)
Milk cortisol (n=31)
Infant behavior diary (n=14)
Infant's weight & BMI (n=31)
HV 2
Infant's age: 6-8 weeks
Mother-infant dyad (n=32)
Maternal stress & anxiety (n=31)
Milk cortisol (n=30)
Infant behavior diary (n=23)
Infant's weight & BMI (n=32)
Mother-infant dyad (n=31)
Maternal stress & anxiety (n=30)
Milk intake (isotope data) (n=11)
Infant's weight & BMI (n=31)
HV 3
Infant's age: 12-14
Mother-infant dyad (n=32)
Maternal stress & anxiety (n=31)
Milk intake (isotope data) (n=8)
Infant's weight & BMI (n=32)
Mother-infant dyad (n=30)
Infant's weight & BMI (n=30)
Infant's body composition
(isotope data) (n=12)
HV 4
Infant's age: 14-18
Mother-infant dyad (n=32)
Infant's weight & BMI (n=32)
Infant's body composition
(isotope data) (n=17)

FIGURE 1

Flow diagram of participants from Phase 1 (antenatal period) to Phase 2 (home visits during the postnatal period) of MOMS trial. The majority of women (n=128) that were ineligible to participate in Phase 1 were multiparous and/or planned to stay outside the study area (Klang-Valley) during the postnatal period. Two mothers were lost to follow-up: 1 person from RG at HV2 and another person from CG at HV4 due to work commitments. Two mothers from RG discontinued intervention starting at HV2 due to stopping breastfeeding (n=1) (hence not able to provide breast milk samples for milk cortisol analysis at HV2) and being unable to continue due to work commitments (n=1). Incomplete isotope analyses at HV1 and HV3 was due to the involvement of a third party during storage (for milk intake data), and implausible results from the IRMS analyses (for infant body composition). Compliance with completion of the 3-day diary was not high at HV1 and HV2, which could be due to the large number of different tasks that mothers were asked to perform over the study period (see further explanation in the discussion section). Almost all mothers completed stress and anxiety questionnaires at HV1-3 and all infants were measured for weight and height at all HVs.