

## Exclusive breastfeeding up to six months: Are we getting the right figures?

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### Abstract

**Introduction:** Exclusive breastfeeding (EBF) is recommended up to six months of age and is usually estimated by the 24-recall method which actually overestimates the real rate. EBF rate in Sri Lanka up to 4 months of age is 75% and up to 6 months of age is 51% according to data of the Sri Lanka Demographic & Health Survey 2006/07. Deuterium oxide dose-to-mother technique could be utilised for assessment of intake by babies of breastmilk as well as intake of water from sources other than breastmilk.

**Objective:** To determine the actual EBF rates at 3 and 6 months of age of infants in Sri Lanka using deuterium oxide dose-to-mother technique.

**Method:** Thirty healthy mother-infant dyads were followed up for a six month period from birth and breast milk intake was measured at 3 and 6 months using deuterium isotope analysis. Further, an interviewer administered questionnaire asked about the feeding history.

**Results:** The average milk intake at 3 months was 772±134 g/day<sup>1</sup> (range 587-1057) and 800±174 g/day<sup>1</sup> (range 500-1113) at 6 months ( $p=0.30$ ). The non-milk oral intake at 3 months was 91±45 g/day<sup>1</sup> (range -2-166) and 128±63 g/day<sup>1</sup> (range 25-304) at 6 months ( $p=0.01$ ). Breastfeeding practices revealed that only 40% ( $n=12$ ) of mothers at 3 months and 30% ( $n=9$ ) of mothers at 6 months were practising EBF. It confirmed that the EBF rate

among study subjects was 50% at 3 months and 27% at 6 months, with the cut-off of 82.5 g/day<sup>1</sup> of non-milk oral intake.

**Conclusions:** A decrease in EBF was noted between 3 and 6 months using deuterium oxide dose-to-mother technique. Further, the EBF rates in this study sample are much lower than the presently reported value for Sri Lanka using recall method.

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(Key words: Exclusive breastfeeding, deuterium oxide dose-to-mother, milk intake, non-milk oral intake, body composition, fat mass)

### Introduction

World Health Organization recommends exclusive breastfeeding (EBF) for the initial six months of life, followed by appropriate complementary foods and continuing breastfeeding till two years of age to achieve optimal growth, development and health<sup>1</sup>. Multiple studies have been conducted on the macro- and micro-nutrient composition of human milk throughout the different stages of lactation at various geographical, ethnic and socio-economic settings across the globe<sup>2,3</sup>. However, there is very little information on the quantity of breastmilk consumed by infants, especially in developing countries<sup>4</sup>.

Accurately measuring EBF duration is complicated by factors associated with definitions, timing, recall duration, analytic methods and sample bias<sup>5</sup>. The deuterium oxide dose-to-mother technique is more accurate than the conventional methods, can be applied in the field setting and is safe for both mother and baby<sup>4</sup>. In this technique most of the non-deuterated water percentage is equal to the additional liquid intake of the infant.

### Objective

To determine the actual EBF rates at 3 and 6 months of age of infants in Sri Lanka using deuterium oxide dose-to-mother technique.

### Method

Randomly selected 30 mother-baby pairs with mothers' age <40 years, following the 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> pregnancy with a healthy term single baby were recruited for the study. Study protocol was approved by the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka, and institutional approvals were obtained


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The authors declare that there are no conflicts of interest

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from the Ministry of Healthcare and Nutrition and the Regional Director of Health Services, Galle. Inclusion criteria were mothers of 18-40 years of age, no more than 3 parities and having term baby (37-40 weeks) and who would practise ERF for 6 month period. Twin or pre-term or babies with complicated deliveries were excluded. Written informed consent was obtained from the mothers after explaining the procedures, expected outcomes, and the value of the research. The questionnaire on EBF was administered to be validated against the deuterium dose to mother method to assess breast milk intake of babies aged 3 and 6 months among participating countries. The sample of 30 mother-baby dyads can detect a difference in the proportion of true non-exclusive breastfeeding mothers (EBF defaulters) of 0.6. For example, if the proportion of defaulters by questionnaire is  $<0.1$  and proportion of defaulters by dose to mother is 0.7, the sample of 30 will suffice to demonstrate a significant difference. Basal saliva samples (by asking the mother to roll sterile cotton wool under the tongue for 1-2 minutes until the cotton is soaked with saliva and placing a sterile cotton wool under the baby's tongue using a stick for about 30-40 seconds) and anthropometry were taken from both prior to dosing with deuterium oxide ( $D_2O$ ) to the mother (i.e. day 0). Soaked swab/ cotton wool was placed in a 10 ml syringe under hygienic conditions and expressed into the vial to obtain 1 mL of saliva in duplicate. Once the basal saliva sample was collected, an oral dose of 30g deuterated water (i.e.  $D_2O$ ) was administered to mother. Then further saliva samples were collected (post-dose) on days 1, 2, 3, 4 and 13, 14, from both mother and infant. Samples were frozen at  $-80^{\circ}C$ , stored, and transported to the USDA/Agricultural Research Service Children's Nutrition Research Centre at

Baylor College of Medicine in Houston, Texas, for isotope analysis.

Intake of breast milk was calculated following the method described previously<sup>6</sup>. Curves were generated using data obtained from isotope levels of saliva samples and the time elapsing after administering  $D_2O$  to mothers, to reflect the disappearance of isotope from mothers' body water and its appearance and disappearance from infants' body water. The curves were fitted to the equations using the Solver function of Microsoft Excel. From the fitted curves, information regarding the mother's body composition and daily water intake was determined. This methodology calculates the mean breast milk intake over a 2-week period.

Major/primary outcome variable of study was the proportion of infants exclusively breastfed. Secondary outcome variables were volume of breast milk intake and non-milk oral intake. Data on two-time points (3 and 6 months) were confirmed with normal distribution and compared with paired t-test and any result was considered as significant at 5% level of significance. Microsoft Excel 2016 was used for data entry and analysis.

### Results

The age of mothers at 3-months lactation was  $29.8 \pm 4$  years. Ten mothers were from first pregnancy, 15 on their second baby and the remainder ( $n=5$ ) had 2 live births on earlier occasions. Only 3 mothers were employed, and they were on maternity leave during study period (i.e. 6 months). The mean birth weight and mean weights at 3 and 6 months of study subjects were  $2.9 \pm 0.4$ kg,  $5.3 \pm 0.7$ kg,  $6.5 \pm 0.7$ kg respectively. Kinetic data of infants and mothers based on the deuterium oxide-to-the-mother model are shown in Table 1.

**Table 1: Kinetic data of the mothers and infants based on the deuterium-oxide-to-the-mother model (n=30)**

Kinetic data (unit)	3 months Mean ± SD	6 months Mean ± SD	p value*
<b>Mother</b>			
Deuterium dilution space (kg)	30.6±4.5	30.9±4.4	0.45
Total body water (kg)	29.4±4.3	29.7±4.2	0.45
Fat-free mass (kg)	40.2±5.9	40.6±5.7	0.45
Body fat (kg)	14.0±6.0	13.1±6.5	0.08
Fat mass (%)	24.9±7.0	23.2±8.4	0.08
<b>Infant</b>			
Human milk intake (g.d <sup>-1</sup> )	770±140	803±170	0.22
Water input from milk (g.d <sup>-1</sup> )	740±130	772±170	0.22
Water used in growth (g.d <sup>-1</sup> )	10±4	5±6	<0.001
Total water output (g.d <sup>-1</sup> )	878±140	956±180	<0.001
Non-oral water intake (g.d <sup>-1</sup> )	57±10	61±10	0.04
Non-milk oral intake (g.d <sup>-1</sup> )	94±40	129±60	0.01
**Square root MSE (mg/kg <sup>-1</sup> )	12.065	8.720	0.22

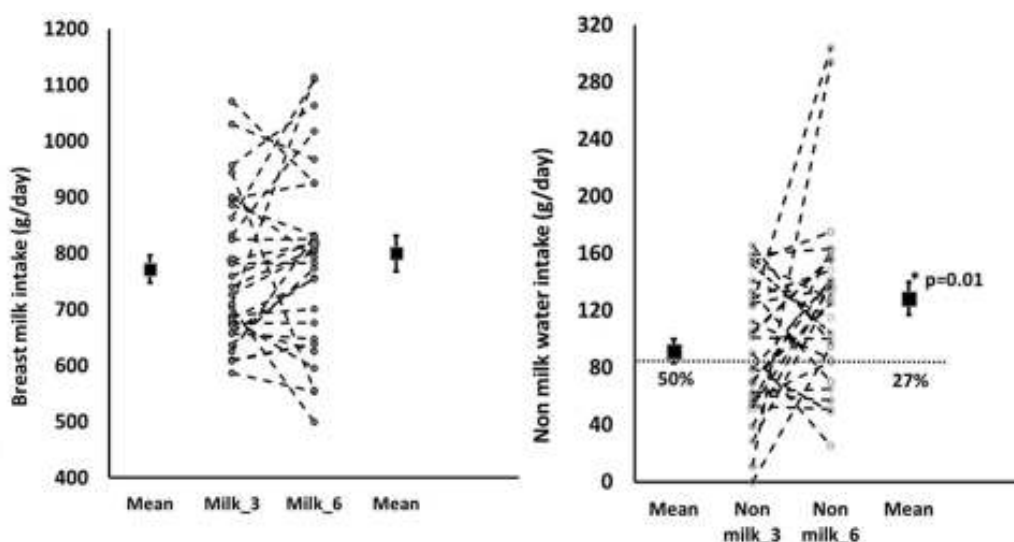
\*Based on t-test: paired two sample for means at probability level of 0.05

\*\*MSE is the square of the differences between the measured and predicted deuterium enrichment in the mother and in the infant. The differences are squared to get rid of negative values. The square root of the sum of the MSE of the mother and the MSE of the infant represents the total error of the model and reflects how well the data are fitting the model.

No significant difference was found in any kinetic parameter on maternal body composition. In the infant, human milk intake and the water input from milk was not different at the two intervention points. However, the non-milk (oral) intake and non-oral water intake significantly increased with age (p=0.04 and p=0.02 respectively). The square root of the mean square error (expressed as mg/kg<sup>1</sup>) of the model was very small, indicating a good fit of data to the model.

Information on the breastfeeding practices revealed that only 40% (n=12) at 3-months and 30% (n=9) of mothers at 6-months were practising EBF. The

other mothers (n=18 at 3-months and 21 at 6-months) admitted giving either water or water with sugar or formula during this period. The average milk intake at 3-months was 772±134 g/day<sup>1</sup> (range 587 -1057) and 800±174 g/day<sup>1</sup> (range 500 – 1113) at 6-months (p=0.30) respectively. The non-milk oral intake at 3-month was 91±45 g/day<sup>1</sup> (range -2 –166) and 128±63 g/day<sup>1</sup> (range 25 – 304) at 6-months respectively (p=0.01). When the cut-off of 82.5 g/day<sup>1</sup> of non-milk oral intake applied we found that the EBF rate among study subjects were 50% at 3-months (n=15) and 27% at 6-months (n=8) respectively (Figure 1).



**Figure 1: Breast milk intake at 3 months and 6 months among each study participant (left panel) and non-milk oral intake (right panel) using deuterium isotope analysis**

\*The dash line in right panel indicates the cut-off value (82.5g.d<sup>-1</sup>) for non-milk oral intake. The Mean ± SEM was indicated at each investigation point.

Breastmilk intake was unaffected by mother’s age at delivery (p=0.13), parity (p=0.50), body weight

(p=0.69), body mass index (p=0.26), fat free mass (p=0.72), or % fat mass (p=0.80). However, milk

intake was positively associated with baby's weight ( $p=0.01$ ,  $r=0.59$ ) at 3 months of lactation. Milk intake was also positively related to baby's age ( $p=0.001$ ,  $r=0.57$ ) and negatively to mother's age ( $p=0.03$ ,  $r=-0.40$ ) at 6 months of lactation.

### Discussion

This is the first ever longitudinal study to assess the breastfeeding during the recommended period of six months of life among infants in Sri Lanka. The average breast milk intake at 3 and 6 months of life is comparable with previously published data from elsewhere<sup>7,8</sup>. In a previous study we reported that the EBF rate for first 6 months among Sri Lankan mothers was 49.0%, based on mother's recall<sup>9</sup>. When a lactating mother drinks a dose of D<sub>2</sub>O which is distributed throughout her body in a short time (incorporated into her milk), baby receives 2H only during breast-feeding. Saliva of both mother and child is enriched with 2H which can be measured by an isotope ratio mass spectrometer and the alterations in isotope concentration show the amount of breast milk consumed by baby and whether baby has consumed water from other sources. With this objective assessment we were able to reveal the actual EBF rate to be 27% at 6 months in this group of mothers.

Deuterium is a naturally occurring stable isotope of hydrogen. It does not decay or emit any harmful radiation as compared to radioactive isotopes such as tritium or carbon-14. Deuterium has a natural abundance of 0.0156% and is found in our body tissues (in a 50-kg adult, there is approximately 1.5g of deuterium). Numerous studies have been carried out using deuterium to measure energy expenditure, body composition and breast milk consumption in thousands of premature infants, new-borns, toddlers, adolescents, pregnant teens, pregnant and lactating women, and older minority women with no adverse effects<sup>4</sup>.

The WHO recommended EBF definition is based on 24-hour dietary recall and can be used only for infants of 0-6 months old. This is the method used in the Sri Lanka Demographic and Health Survey and is the basis of the reported EBF rate of 75% in Sri Lanka<sup>10</sup>. 24-hour recall method over-estimates EBF prevalence as the data are based only on feeding practices for the day previous to the survey. Infants infrequently given liquids or foods, but not given during the day prior to the survey, are classified as exclusively breastfed. Labbock defined EBF rate as "the proportion of infants less than six months of age who received only breast milk". For this definition, either it must be a cohort study, or the investigator must obtain a detailed history of feeding practices from the time of birth<sup>11</sup>. The since-birth recall methods also overestimated EBF rates because of the difficulty in correctly

remembering practices occurring long ago. Further, previous studies revealed that mothers' own recall of EBF overestimates it by around 40% at 3-6 months of age<sup>12</sup>.

Our results agree with the reported values on mean human milk intake of well-nourished mothers (~700-800 g/day<sup>1</sup>). Further, we believe that the mothers in this study have answered truthfully as they knew that it will be checked biochemically. We recruit subjects with a written agreement of EBF for 6 months period. Yet, they did not comply with such assurance. Excessive crying of baby and pressure from other family members (i.e., grandmother, father or relative of the baby) are the main excuses given by mothers who initiate feeding other than breast milk during this period. This fact raises concerns regarding the accuracy of high EBF rates based on maternal recall collected in surveys. Mothers may be giving other fluids without admitting it in routine surveys in fear of being reprimanded by health care workers.

We suggest conducting objective assessments using deuterium to mother technique to be used in conjunction with routine surveys to assess EBF rates accurately while consolidating and reviewing present breastfeeding promotion and education programmes at least in selected areas.

### Conclusions

A decrease in EBF was noted between 3 and 6 months using deuterium oxide dose-to-mother technique. Further, the EBF rates in this study sample are much lower than the presently reported value for Sri Lanka using recall method.

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