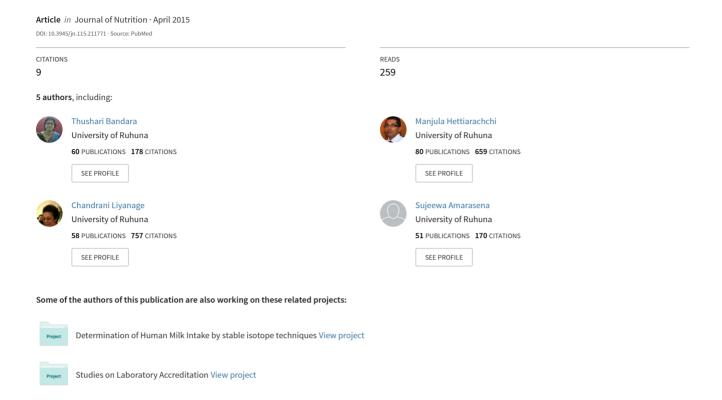
## The Deuterium Oxide-to-the-Mother Method Documents Adequate Breast-Milk Intake among Sri Lankan Infants



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# The Deuterium Oxide-to-the-Mother Method Documents Adequate Breast-Milk Intake among Sri Lankan Infants<sup>1-4</sup>

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#### **Abstract**

**Background:** The WHO recommends that exclusive breastfeeding should last up to 6 mo. However, human milk intake of Sri Lankan infants has not been quantified scientifically.

**Objectives:** The objectives of this study were to measure the human milk intake of Sri Lankan infants during the first 6 mo of age and to document the breastfeeding practices of their mothers.

**Methods:** Forty-eight healthy mother-infant dyads were randomly recruited for this cross-sectional study at well-baby clinics. Milk intake was measured using the deuterium oxide—to-the-mother technique over a period of 2 wk. Information on breastfeeding practice and living standards of the participants were gathered using an interviewer-administered questionnaire

**Results:** Human milk intake was  $672 \pm 123 \text{ g} \cdot \text{d}^{-1}$  (mean  $\pm$  SD),  $776 \pm 212 \text{ g} \cdot \text{d}^{-1}$ , and  $801 \pm 51 \text{ g} \cdot \text{d}^{-1}$  for infants <2 mo, 2 to <4 mo, and 4–6 mo of age, respectively. The milk intakes were not different among the age groups. Maternal body composition, age, or parity had no effects on milk intake of the infants. However, mother's education (P < 0.05, r = 0.35), infant's age (P < 0.05, r = 0.30), and body mass index (P < 0.05, r = 0.41) positively correlated with the milk intake. Over 63% of mothers had commenced breastfeeding within 30 min of delivery. About 60% of the mothers were feeding the baby 6–10 times during the daytime and >81% intended to continue exclusive breastfeeding until 6 mo of age.

**Conclusion:** This study, for the first time, documented the adequacy of breast-milk intake among Sri Lankan infants and the nutrition status of the mothers. *J Nutr* 2015;145:1325–9.

Keywords: breast-milk production, deuterium oxide, infants, isotope ratio mass spectrometry, Sri Lanka

#### Introduction

Breast milk is the best source of nutrition for infants (1). Exclusive breastfeeding (EBF)<sup>7</sup> reduces the risk of a wide spectrum of illnesses in early childhood (2) with a reported reduction of infant mortality by 13% (3). In addition, EBF contributes to the health and well-being of the mothers. Therefore, the WHO recommends that infants should be

exclusively breastfed during the first 6 mo of life (4). Breastfeeding practices and adequacy of human milk intake among infants in developing countries such as Sri Lanka (5) are limited.

The present study measured the breast-milk intake using the deuterium oxide—to-the-mother technique during the first 6 mo of life among Sri Lankan infants to assess their adequacy of human milk intake and to document the breastfeeding practices of Sri Lankan mothers. The technique further enables the measurements of nonmilk (oral) intake of the infants and body composition of the mothers. The measurement of body composition among lactating mothers in developing countries is of particular importance for identifying intervention strategies in order to improve nutrient intake to support optimal lactation.

#### Methods

Subjects. Forty-eight mother-infant dyads, stratified across 3 age groups (<2 mo, 2 to <4 mo, and 4–6 mo), were randomly selected from the

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<sup>&</sup>lt;sup>4</sup> Supplemental Tables 1 and 2 are available from the "Online Supporting Materials" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

 $<sup>^{7}</sup>$  Abbreviations used: EBF, exclusive breastfeeding; FM, fat mass; TBW, total body water; %FM, percentage of fat mass.

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maternal and child health clinics of the Medical Officer of Health areas in Galle, Sri Lanka. Sixteen infants were included in each age group, enabling the detection of 1 SD change in breast-milk intake between groups at a *P* value of 0.05 and a power of 0.8. All infants must be EBF in order to participate in the study. Before enrollment, eligibility of the mother-infant pairs was checked using a brief demographic and health questionnaire. Mothers who had complicated pregnancies and infants with any congenital anomalies, chronic diseases, or any illness that could affect breastfeeding or growth were excluded from the study.

Study protocol was approved by the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka, and the institutional approvals were obtained from the Ministry of Healthcare and Nutrition and the selected Medical Officers of Health offices. Written informed consent was obtained from the mothers after explaining the procedures, expected outcomes, and the value of the research.

Human milk intake measurement. The deuterium oxide-to-themother technique was used to measure human milk intake over a period of 14 d (6). Sampling was performed according to the procedure of Galpin et al. (7). Mothers (with light clothing) and the infants (nude) were weighed using standard scales. Mothers' weights were measured to the nearest 0.1 kg and infants' to the nearest 5 g. Infant length was measured, using an infantometer, to the nearest 1 mm. Predose samples of the mother's saliva (2 mL) and infant's urine (2 mL) were collected for <sup>2</sup>H analysis by isotope ratio mass spectrometry. On day 1, an accurately measured dose of 10 g D<sub>2</sub>O (99.9% D<sub>2</sub>O; Sigma-Aldrich Corp.) was administered orally to the mother. The mothers remained in the sitting position for the next 5-10 min. Mothers' saliva samples were collected on days 1 (5 h postdose), 4, and 14. The saliva samples were obtained by asking mothers to keep a small sterile cotton ball in her mouth for a few minutes according to the protocol of Haisma et al. (8). The saliva was expressed from the wet cotton wool ball using a 10-mL syringe.

Infants' urine samples were collected at each of the follow-up visits on days 1 (5 h postdose), 3, 4, 13, and 14 postdosing. Urine sampling was performed by keeping sterile cotton balls in the nappies of the babies. The urine was expressed from the wet cotton balls using a 20-mL syringe.

Saliva and urine samples were frozen at -20°C, stored, and transported to the USDA/Agricultural Research Service Children's Nutrition Research Center at Baylor College of Medicine in Houston, Texas, for isotopic analyses. Infants' body weights were measured again on day 14 to monitor growth.

Analysis of deuterium enrichment. The saliva and urine samples were processed for stable hydrogen isotope ratio measurements using a Thermo Delta V Advantage continuous-flow isotope ratio mass spectrometer system (9). The method was accurate to  $2.8\%_{oo}$  (0.41 ppm) and reproducible to within  $4.0\%_{oo}$  (0.60 ppm) based on analysis of international references.

Calculation of breast-milk intake. The method of Haisma et al. (8) was used in the calculation of breast-milk intake. Curves were generated using the deuterium data obtained from the urine and saliva samples and the time elapsed after the administration of the isotope to the mothers. The curves were fitted to the following equations using the Solver function in Microsoft Excel. From the fitted curves, information regarding the mother's body composition, daily water intake, and mean breast-milk intake of >2 wk were calculated.

Equation for mothers: 
$$E_{m(t)} = E_{m(0)}e^{-Kmm-t}$$
 (1)

where  $E_{m(t)}$  is isotope enrichment of a sample at time t (expressed as  $\text{mg} \cdot \text{kg}^{-1}$ );  $E_{m(0)}$  is isotope enrichment of the sample at time 0 (expressed as  $\text{mg} \cdot \text{kg}^{-1}$ ); t is time elapsed since administration of the isotope (expressed as d);  $K_{mm}$  is water turnover in the mother (expressed as  $\text{g} \cdot \text{d}^{-1}$ ).

Equation for infants: 
$$E_{b(t)}=E_{m(0)}(F_{bm}/V_b)\Big(e^{-Kmm-t}-e^{(Fbb/Vb)t}\Big)/\\ \Big[(F_{bb}/V_b)-K_{mm}\Big]$$
 (2

where  $E_{b(t)}$  is isotope enrichment of the sample taken at time t (expressed as  $\text{mg} \cdot \text{kg}^{-1}$ );  $E_{m(0)}$  is isotope enrichment of the sample at time 0

(expressed as  $\operatorname{mg} \cdot \operatorname{kg}^{-1}$ );  $F_{bm}$  is flow of water from the mother to the baby via breast milk (expressed as  $\operatorname{kg} \cdot \operatorname{d}^{-1}$ );  $V_b$  is infant's distribution space (expressed as kg);  $K_{mm}$  is water turnover in the infant (expressed as  $\operatorname{g} \cdot \operatorname{d}^{-1}$ );  $F_{bb}$  is total water loss in the baby (expressed as  $\operatorname{kg} \cdot \operatorname{d}^{-1}$ ).

This method is based on the assumption that  $V_b$  is changed linearly with weight over the course of the study period and is related to the infant weight (W) in kilograms by  $V_b = 0.84 \, \mathrm{W}^{0.82}$ .

Breastfeeding practices. Sociodemographic characteristics and breastfeeding practices of each mother-infant pair were collected using a pretested, interviewer-administered questionnaire. Pretesting of the questionnaire was performed on 10 mothers, randomly selected, who were not included in the study to ensure the objectivity of data collection.

Statistical analysis. Demographic characteristics were tabulated as mean  $\pm$  SD for continuous variables and as percentage for dichotomous variables. The association between breast-milk intake on demographic and anthropometric characteristics of the subjects was assessed by Pearson correlation. Group comparisons were performed using 1-factor ANOVA post hoc multiple comparison with Bonferroni tests. Univariate ANOVA was used to evaluate the potential effect of gender on group comparisons. All statistical procedures were performed using the Statistical Package for Social Sciences (version 22; SPSS, Inc.) at a P value of 0.05.

#### Results

A total of 48 mother-baby dyads were recruited in the study to represent 3 age groups, viz., <2 mo, 2 to <4 mo, and 4–6 mo of age with 16 infants in each group.

Description of mother-infant dyads. Tables 1 and 2 represent the characteristics of the mother-infant dyads. The age of the mothers was  $27.8 \pm 6.2$  y. Parity was 2 on average. The majority of the mothers (71%) had passed the General Certificate of Examination or achieved higher levels of education. The average monthly income of families was 22,865 Sri Lankan rupees or US \$177.

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Body composition of the mothers. Kinetic data of the mothers are shown in Table 3. No significant differences were found in the total body water (TBW), fat-free mass, fat mass (FM), and percentage of FM (%FM) among the 3 groups of infants. Exclusion of the mothers with low %FM (n = 3, %FM = 1-8) did not change the mean milk intake value or the association between milk intake and maternal body composition.

**Breast-milk intake.** Table 3 also summarizes the kinetic data of the infants. The human milk intake was not different among the 3 groups of infants (P = 0.11). The nonmilk (oral) intake did not increase with age (P = 0.14). The square root of the mean square error (expressed as  $mg^2 \cdot kg^2$ ) of the model was very small, indicative of good fit of data to the model.

Milk intake was not affected by the mother's age (P = 0.13), parity (P = 0.50), body weight (P = 0.69), BMI (P = 0.26), fatfree mass (P = 0.72), %FM (P = 0.80), occupation (P = 0.09), or family income (P = 0.14). However, milk intake was positively related to the mother's education (P = 0.02, P = 0.35). Furthermore, milk intake was positively associated with BMI (P < 0.01, P = 0.41) and age (P < 0.05, P = 0.30) of the infant.

*Infants' growth.* Infants' milk intake, weight gain, weight-forage z scores, length-forage z scores, and ponderal index by gender are shown in **Table 4**. After normalization of the milk

**TABLE 1** Maternal characteristics<sup>1</sup>

Characteristic	Value
Weight, kg	
<2 mo	51.2 ± 8.6
2 to <4 mo	$55.9 \pm 9.9$
4–6 mo	$46.9 \pm 12.9$
Height, m	
<2 mo	$1.52 \pm 0.06$
2 to <4 mo	$1.54 \pm 0.04$
4–6 mo	$1.51 \pm 0.06$
BMI, kg/m <sup>2</sup>	
<2 mo	$22.2 \pm 3.3$
2 to <4 mo	$23.4 \pm 3.9$
4–6 mo	$20.4 \pm 4.9$
Ethnicity, n (%)	
Sinhalese	47 (97.9)
Tamil	1 (2.1)
Age, n (%)	
<20 y	4 (8.3)
20–30 y	26 (54.2)
>30 y	18 (37.5)
Parity, n (%)	
1	19 (39.6)
2	16 (33.3)
≥3	13 (27.1)
Education, <sup>2</sup> n (%)	
Up to grade 5	14 (29.2)
Passed O/L	19 (39.6)
Advanced level and above	15 (31.2)
Employment, n (%)	
Unemployed	44 (91.7)
Employed	4 (8.3)
Income, <sup>3</sup> n (%)	
<10,000 Sri Lankan rupees	5 (10.4)
10,000–20,000 Sri Lankan rupees	24 (50.0)
>20,000 Sri Lankan rupees	19 (39.6)
Mode of delivery, n (%)	
Normal vaginal delivery	33 (68.8)
Caesarian section	15 (31.2)

<sup>&</sup>lt;sup>1</sup> Values are n (%) or means  $\pm$  SDs, n = 16. GCE, General Certification Examination; O/L. Ordinary Level.

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intake by body weight, the <2-mo-old infants had the highest milk intake compared with the 2- to <4-mo-old infants (P = 0.02) and the 4- to 6-mo-old infants (P < 0.05). There was no difference in the weight normalized milk intakes between the 2- to <4-mo-old infants and the 4- to 6-mo-old of infants (P = 0.23). Weight gain was the highest among the <2-mo-old infants compared with the 2- to <4-mo-old infants (P < 0.05) and the 4- to 6-mo-old infants (P < 0.05). Weight gain of the 2- to <4-mo-old infants also was higher than that of the 4- to 6-mo-old infants (P = 0.01). Gender had no effect on milk intake (P = 0.16) or weight gain (P = 0.21). Age and gender had no effects on the weight-for-age z scores (P = 0.58), length-forage z scores (P = 0.90), and ponderal index (P = 0.14).

Breastfeeding practices and living indices. Information on the breastfeeding practices of the mothers is summarized in Supplemental Table 1. Over 62% of the mothers had commenced breastfeeding within 30 min of the delivery. About 60.4% of the mothers said they breastfed their babies about 6-10 times during the day and 35.4% breastfed their babies >6 times at night. About 58.3% of the mothers were feeding their babies from 1 breast until they stopped sucking. Only 33.3% of the mothers were reported to practice on-demand feeding. Over 95% of the mothers were satisfied with breastfeeding and 81.2% of the mothers intended to continue EBF until 6 mo of age.

Participants' living standards are also summarized in Supplemental Table 1. Among the participants, >93% lived in their own houses with >95% of the houses of pucca type. All participants had a separate kitchen in their houses. The majority (89.6%) was using firewood as the cooking fuel and the rest were using biogas. About 98% of the families used electricity for lighting. All participants had pure water to drink and satisfactory sanitary facilities.

#### **Discussion**

Breastfeeding has been a long time-honored tradition and a norm in Sri Lanka (1). Since 2005, the Ministry of Healthcare and Nutrition of Sri Lanka has extended its policy on the duration of EBF up to 6 mo. During 2006–2007, 75.8% of Sri Lankan infants who were up to 5 mo of age were EBF, representing the highest in the Southeast Asian region (10). However, the research findings during the past few years at different locations in Sri Lanka have shown less EBF rates (11–15).

All of these studies have determined the EBF rates by either the 24-h recall or the since-birth recall methods. However, both methods substantially overestimate the prevalence of EBF (16). Hence, the determination of human milk intake by a more objective approach other than conventional surveys is of paramount importance. Quantification of human milk intake is useful for estimation of adequacy of nutrient intake via breast milk in EBF infants (17).

**TABLE 2** Infant characteristics<sup>1</sup>

	Value			
Characteristic	Male	Female		
Weight, kg				
<2 mo	$3.87 \pm 0.69$	$3.98 \pm 0.48$		
2 to <4 mo	$5.84 \pm 0.58$	$4.96 \pm 0.48$		
4–6 mo	$6.81 \pm 0.90$	$6.30 \pm 1.01$		
Length, m				
<2 mo	$0.55 \pm 0.02$	$0.56 \pm 0.02$		
2 to <4 mo	$0.63 \pm 0.02$	$0.59 \pm 0.02$		
4–6 mo	$0.65 \pm 0.03$	$0.64 \pm 0.03$		
Gestational age, wk				
<2 mo	$39.0 \pm 1.2$	$39.8 \pm 0.7$		
2 to <4 mo	$38.9 \pm 1.4$	$38.8 \pm 1.3$		
4–6 mo	$39.0 \pm 1.2$	$39.0 \pm 1.2$		
Birth weight, kg				
<2 mo	$2.95 \pm 0.34$	$3.25 \pm 0.43$		
2 to <4 mo	$3.07 \pm 0.29$	$2.59 \pm 0.46$		
4–6 mo	$3.07 \pm 0.58$	$2.75 \pm 0.43$		
Weight gain, g/2 wk				
<2 mo	531 ± 131	409 ± 148		
2 to <4 mo	$286 \pm 135$	$276 \pm 139$		
4–6 mo	128 ± 96	$158 \pm 77$		

<sup>&</sup>lt;sup>1</sup> Values are means  $\pm$  SDs, n = 8 (except at 4–6 mo: males, n = 7; females, n = 9).

<sup>&</sup>lt;sup>2</sup> To qualify to study for the university entrance examination, the students must pass the O/L of the GCE at grade 11. After 2 y of study, the students must pass the Advanced Level of the GCE to be considered for admission to a university.

<sup>3</sup> US\$1 = 129 Sri Lankan rupees

**TABLE 3** Kinetic data of the mothers and the infants based on the deuterium oxide-to-the-mother model<sup>1</sup>

Index	<2 mo	2 to $<$ 4 mo	4–6 mo	
Kinetic data of the mothers				
Deuterium dilution space, kg	29.1 ± 3.6 (24.4–37.4)	$36.1 \pm 3.8 (25.2-37.3)$	27.8 ± 4.9 (20.5–42.0)	
TBW, kg	$28.0 \pm 3.4 (23.5-35.9)$	$29.9 \pm 3.7 (24.2-35.9)$	$26.7 \pm 4.7 (19.7-40.3)$	
Fat-free mass, kg	$38.2 \pm 5.9 (32.0-49.1)$	$40.8 \pm 5.0 (33.0-49.0)$	$36.5 \pm 6.4 (26.9-55.1)$	
FM, kg	$12.6 \pm 5.0 (5.5-22.6)$	15.9 ± 5.2 (8.4–27.1)	$11.7 \pm 6.3 (4.5-28.2)$	
%FM	$24.0 \pm 6.2 (13.4-33.2)$	$27.4 \pm 4.8 (19.4-35.6)$	$23.0 \pm 6.7 (12.1-35.0)$	
Deuterium concentration in mother's body water at time 0, mg $\cdot$ kg $^{-1}$	$354 \pm 41 (251-416)$	$331 \pm 42 (273-403)$	$375 \pm 57 (244-498)$	
Fractional water turnover rate in the mother, $g \cdot d^{-1}$	$144 \pm 33 (110-220)$	182 ± 174 (120–810)	$153 \pm 27 (120-220)$	
Transfer rate of water from mother to baby via human milk, $g \cdot d^{-1}$	585 ± 112 (410-790)	674 ± 192 (232–950)	699 ± 137 (400-990)	
Kinetic data of the infants				
Human milk intake, $g \cdot d^{-1}$	672 ± 127 (474–906)	773 ± 219 (266–1089)	802 ± 156 (465-1134)	
Water intake from milk, $g \cdot d^{-1}$	646 ± 122 (455–871)	$743 \pm 211 (256-1046)$	771 ± 150 (447–1090)	
Water used in growth, $g \cdot d^{-1}$	$19 \pm 6^{a} (8-27)$	$11 \pm 5^{b} (0-20)$	$6 \pm 3^{c} (-2 \text{ to } 12)$	
Total water output, $g \cdot d^{-1}$	$703 \pm 116^{a} (515-899)$	$859 \pm 162^{b} (576-1101)$	931 ± 183 <sup>b</sup> (699–1411)	
Total water loss in the baby, $g \cdot d^{-1}$	$700 \pm 110^{a} (510-890)$	$850 \pm 160^{b} (570-1090)$	$920 \pm 180^{b} (690-1400)$	
Nonoral water intake, $g \cdot d^{-1}$	$45 \pm 8^{a} (33-58)$	$55 \pm 10^{b} (37-70)$	$59 \pm 12^{b} (44-89)$	
Nonmilk oral intake, $g \cdot d^{-1}$	$30 \pm 36 (-21 \text{ to } 112)$	$73 \pm 102 (-2 \text{ to } 388)$	107 ± 143 (8-500)	
Square root MSE, 2 mg · kg <sup>-1</sup>	$6.8 \pm 3.7 (1.8-16.0)$	$7.8 \pm 8.8 (1.3-38.1)$	$6.5 \pm 4.3 (1.2-15.4)$	

<sup>&</sup>lt;sup>1</sup> Values are means  $\pm$  SDs (range), n = 15. Labeled means in a row without a common letter differ, P < 0.05. Three mothers with %FM < 8% were excluded from the analyses. The low percentage is considered unphysiologic in healthy adult women. FM, fat mass; MSE, mean square error; TBW, total body water; %FM, percentage of fat mass. <sup>2</sup> 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.

This study was a cross-sectional study conducted in rural and urban areas of the Galle district in Sri Lanka. As per standards of living and monthly income, the majority of the participants represented the middle socioeconomic class.

Across country comparison of the human milk intake by the deuterium oxide–dose-to-the-mother method is summarized in **Supplemental Table 2.** Human milk intake of the Sri Lankan infants appears to be moderate in comparison with the values reported in other countries (8, 16, 18–23). However, according to Rattigan et al. (24), the maximum amount of milk production by a lactating mother ranges between 700 and 900 mL  $\cdot$  d<sup>-1</sup>. Dewey et al. (25) reported that the mean human milk intake of the EBF infants of the well-nourished mothers is ~700–800 g  $\cdot$  d<sup>-1</sup> during the first 5 mo of life. Our results agree with these reported values indicating that Sri Lankan infants who are exclusively breastfed during the first 6 mo of life receive adequate nutrition for growth.

Weight gain of the Sri Lankan EBF infants in the present study is comparable with that reported among EBF infants in the United States  $[36.2 \pm 11.5 \text{ g} \cdot \text{d}^{-1} \text{ vs. } 36.6 \pm 13.6 \text{ g} \cdot \text{d}^{-1} \text{ at } 1 \text{ mo and } 11.2 \pm 6.5 \text{ g} \cdot \text{d}^{-1} \text{ vs. } 12.2 \pm 5.0 \text{ g} \cdot \text{d}^{-1} \text{ at } 4 \text{ mo}]$  (19).

A similar study conducted in Kenya reported an average weight gain of  $20 \pm 4 \text{ g} \cdot \text{d}^{-1}$  in EBF infants at 2–4 mo of age (22).

Nonmilk (oral) intake of the infants did not increase with age. The deuterium oxide–to-the-mother technique categorizes the infants as EBF if the nonmilk oral intake is <25 g · d<sup>-1</sup> (8, 16). In the present study, only 18 infants (37.5%) were found to have nonmilk intake <25 g · d<sup>-1</sup>. However, the nonmilk intake estimated by the deuterium oxide–to-the-mother method is known to have a huge error (SD of 62 g · d<sup>-1</sup>) (5). Therefore, the use of a fixed value of 25 g · d<sup>-1</sup> on nonmilk intake might not be the most appropriate cutoff to define EBF when using the deuterium oxide–to-the-mother method.

The BMI of the lactating mothers in the study was  $22.0 \pm 4.2$  kg·m², which is in the desirable range reflecting satisfactory maternal nutritional status. The %FM of the lactating mothers was  $24.8 \pm 6.1$ %. Haisma et al. (8) in 2003 reported the %FM of the Brazilian mothers at 4 mo of lactation to be  $24.7\% \pm 3.9\%$ . According to Galpin et al. (7), the %FM of Malawi mothers at 6–9 mo of lactation ranged from 22% to 25%. TBW of the mothers in the present study was  $28.2 \pm 4.1$  kg. Butte et al. (18) reported  $31.2 \pm 3.3$  kg of TBW in American mothers

**TABLE 4** Human milk intake and growth of the infants by gender<sup>1</sup>

	<2 mo		2 to <4 mo		4–6 mo	
Variables	Girls ( <i>n</i> = 8)	Boys (n = 8)	Girls ( <i>n</i> = 8)	Boys (n = 8)	Girls ( <i>n</i> = 9)	Boys ( <i>n</i> = 7)
Human milk intake, $g \cdot kg^{-1} \cdot d^{-1}$	165 ± 30°	179 ± 22ª	140 ± 47 <sup>b</sup>	146 ± 17 <sup>b</sup>	135 ± 17 <sup>b</sup>	111 ± 29 <sup>b</sup>
Weight gain, g $\cdot$ d $^{-1}$	$31.5 \pm 11.4^{a}$	$40.9 \pm 10.1^{a}$	$21.3 \pm 10.7^{b}$	$22.0 \pm 10.3^{b}$	$12.2 \pm 6.0^{c}$	$9.8 \pm 7.4^{c}$
Weight-for-age z score	$-0.66 \pm 0.64^{a}$	$-0.58 \pm 0.96^{a}$	$-0.66 \pm 0.64^{a}$	$-0.34 \pm 0.72^{a}$	$-0.28 \pm 0.91^{a}$	$-0.36 \pm 0.95^{a}$
Length-for-age z score	$0.95 \pm 1.16^{a}$	$-0.21 \pm 1.13^{a}$	$-0.15 \pm 0.80^{a}$	$0.65 \pm 0.81^{a}$	$0.16 \pm 0.85^{a}$	$0.28 \pm 0.97^{a}$
Ponderal index, <sup>2</sup> g · cm <sup>3</sup>	$2.32 \pm 0.07^{a}$	$2.28 \pm 0.19^{a}$	$2.38 \pm 0.12^{a}$	$2.35 \pm 0.13^{a}$	$2.44 \pm 0.32^{a}$	$2.45 \pm 0.26^{a}$

 $<sup>^{1}</sup>$  Values are means  $\pm$  SDs. Labeled means in a row without a common letter differ, P < 0.05 (a > b > c).

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<sup>&</sup>lt;sup>2</sup> Ponderal index (g  $\cdot$  cm<sup>3</sup>) = 100  $\times$  weight (g)/[length (cm)]<sup>3</sup>.

at 3–4 mo of lactation using the same technique. In another study, TBW was found to range between 27.2  $\pm$  2.9 kg and 28.5  $\pm$  1.9 kg in well-nourished Mexican mothers at 4-6 mo of lactation (26).

The positive correlation of the milk intake with the mothers education postulates that with higher education, the mothers might be more aware of the health benefits associated with breastfeeding and are more prone to breastfeed their infants and allow them to feed as long as possible. The positive associations of breast-milk intake with the infant's age and BMI are anticipated. With age, infants might get accustomed to proper sucking and positioning while feeding, which may lead to higher milk intake. Haisma et al. (8) also reported positive associations of milk intake with weight-for-age and weight-for-height indexes among 4-mo-old Brazilian infants.

In conclusion, human milk intake of EBF Sri Lankan infants during their first 6 mo of life is adequate when compared with values reported in other developing countries and developed countries. Although 100% of the mothers in the study assured us that they exclusively breastfed their babies, only 37.5% of the babies were found to have  $<25 \text{ g} \cdot \text{d}^{-1}$  of nonmilk intake based on the deuterium oxide-to-the-mother technique. Because the nonmilk intake was derived indirectly from the kinetic model of the isotope method and is known to have a large measurement error, the cutoff at 25 g · d<sup>-1</sup> of nonmilk intake could easily misclassify an infant's breastfeeding status.

Our results also showed that the %FM of the Sri Lankan lactating mothers is comparable with those reported elsewhere using similar methodology (7, 8), indicating that they were adequately nourished.

We recruited mothers for the study at the well-baby clinics. Thus, our study population can be reflective of the mothers who are more concerned about the nutrition and well-being of their infants. However, it is suggested that the maternal and healthcare professionals of Sri Lanka need to investigate the existing breastfeeding promotion and education programs in their areas with a view to improving them in order to minimize the potential high economic costs associated with inadequate breastfeeding and to maximize the potential benefit of breastfeeding on early neurocognitive development.

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