Use of stable-isotope techniques to validate infant feeding practices reported by Bangladeshi women receiving breastfeeding counseling[1–3]

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ABSTRACT

Background: The World Health Organization recommends exclusive breastfeeding until age 6 mo. Studies relying on mothers' self-reported behaviors have shown that lactation counseling increases both the rate and duration of exclusive breastfeeding.

Objective: We aimed to validate reported infant feeding practices in rural Bangladesh; intakes of breast milk and nonbreast-milk water were measured by the dose-given-to-the mother deuterium dilution technique.

Design: Subjects were drawn from the large-scale Maternal and Infant Nutrition Interventions, Matlab, study of combined interventions to improve maternal and infant health, in which women were randomly assigned to receive either exclusive breastfeeding counseling or standard health care messages. Data on infant feeding practices were collected by questionnaire at monthly visits. Intakes of breast milk and nonbreast-milk water were measured in a subsample of 98 mother-infant pairs (mean infant age: 14.3 wk) and compared with questionnaire data reporting feeding practices.

Results: Seventy-five of the 98 subjects reported exclusive breastfeeding. Mean (±SD) breast milk intake was 884 ± 163 mL/d in that group and 791 ± 180 mL/d in the group reported as nonexclusively breastfed ($P = 0.0267$). Intakes of nonbreast-milk water were 40 ± 80.6 and 166 ± 214 mL/d ($P < 0.0001$), respectively. Objective cross-validation using deuterium dilution data showed good accuracy in reporting of feeding practices, although apparent misreporting was widely present in both groups.

Conclusions: The dose-given-to-the-mother deuterium dilution technique can be applied to validate reported feeding behaviors. Whereas this technique shows that the reports of feeding practices were accurate at the group level, it is not adequate to distinguish between feeding practices in individual infants. *Am J Clin Nutr* 2007;85:1075–82.

KEY WORDS Human milk, breastfeeding, Bangladesh, deuterium dilution

INTRODUCTION

The importance of breastfeeding during the first months of life has led to a World Health Organization (WHO) recommendation that exclusive breastfeeding should be continued until 6 mo of age and that nutritionally adequate, safe, and appropriate complementary foods should be introduced, in conjuction with continued breastfeeding, thereafter (1). This recommendation is supported by a large systematic review of evidence showing that, on a population basis, exclusive breastfeeding to age 6 mo provides for the healthy growth and development of the infant and for the health of the mother (2). Until recently, national policy in Bangladesh recommended that exclusive breastfeeding be continued until the infant reached 5 mo of age, but this policy is currently being considered with a view to the international recommendations. However, a population-based survey using data for 4126 mother-child pairs from the 2004 Bangladesh Demographic Health Survey estimated the median duration of exclusive breastfeeding to be as little as 1.7 mo (3), whereas an earlier study of 1100 lower-middle-class mothers from urban Dhaka reported that the prevalence of exclusive breastfeeding was 15% and that complementary foods were introduced at a median of 1 mo (4). It is relevant that this study found that many mothers misinterpreted the term *exclusive breastfeeding*, believing that it meant feeding both breast milk and water; this misunderstanding may explain the higher recorded rates observed within the national survey.

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In Bangladesh, the WHO/UNICEF's Baby-Friendly Hospital Initiative is the main strategy for breastfeeding promotion (5). However, this initiative primarily targets women who receive prenatal care and then deliver their infants in health care facilities. Because ≈91% of Bangladeshi women have home deliveries (3), messages on breastfeeding promotion fail to reach the vast majority of pregnant women. A randomized controlled trial of community-based peer counseling on exclusive breastfeeding practices in Dhaka found that this counseling significantly increased the prevalence of reported exclusive breastfeeding at infant age 5 mo from 6% in the control group (ie, the group receiving no counseling) to 70% in the intervention group (5). No such data are available from rural Bangladesh.

A recognized problem in assessing the effect of lactation counseling is that the effect is based on mothers' self-reported behavior, and it is a concern that mothers in the intervention group may overreport rates of exclusive breastfeeding (5). Whereas methods such as unannounced observations of mother-child interactions, monitoring of infant weight gain, and monitoring of the duration of lactational amenorrhea can help validate reports, no gold standard method of validation is available. In a recent study from Brazil, Albernaz et al (6) used a stable-isotope dosing method to show that lactation counseling increases breastfeeding duration but does not increase breast milk intakes in infants assessed at 4 mo of age. Because this stable-isotope method also estimates the intake of nonmilk fluids, it could potentially be used to validate mothers' reports of infant feeding practices. The current study was specifically designed to measure breast-milk and nonbreast-milk water intakes by using the dose-given-to-the-mother (dose-to-mother) deuterium dilution technique and to validate reported exclusive breastfeeding in a subgroup of mother-infant pairs from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study in rural Bangladesh.

SUBJECTS AND METHODS

Study population

The study was conducted in the Matlab region of Bangladesh, a rural but densely populated riverine area of Bangladesh situated ≈70 km southeast of the capital, Dhaka. Since 1963, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B; formerly the Cholera Research Laboratory), has conducted a demographic surveillance and health-related research program in the region. In 2001, ICDDR,B initiated the MINIMat study, randomly assigning all pregnant women in Matlab to receive a combination of protein-energy and micronutrient supplements. The prenatal arm of the study was completed in June 2004, by which time a total of 3267 singleton infants had been born in the region. Key findings from the main study will be reported elsewhere. In addition to the prenatal dietary supplementation scheme, all women were randomly assigned either to receive (intervention group) or not to receive (control group) exclusive breastfeeding counseling; all women received the standard health counseling provided by local community health workers. Within the intervention group, women received counseling in 8 sessions: 2 sessions before delivery, 1 session ≤7 d after delivery, and 5 sessions at monthly intervals up to 6 mo after delivery. All women received basic health care messages, as is the usual practice in this region of Bangladesh. Analysis of the effect of this intervention on the rates and duration of exclusive breastfeeding within the main study is ongoing.

Written informed consent was obtained from all participating mothers. Approval for the study was obtained from the Ethical Review Committee of the ICDDR,B Centre for Health and Population Research.

Classification criteria for infant feeding

At monthly visits after delivery, a female fieldworker visited each household to collect information on infant feeding practices. At these visits, the mother was asked to describe how her infant had been fed during the previous 30 d, and data were recorded separately for 2 time periods—the first 15 d and the most recent 15 d. The purpose of using 2 times periods was to capture, as accurately as possible, the age at which the child was no longer exclusively breastfed. Because of the large geographical area covered by the study, and the fact that visits to subjects can involve long distances on foot, bicycle, or boat, more frequent visits were not possible. Information was recorded as to whether the infant had received breast milk only; received breast milk and additional foods including plain water, sugar water, other milk, other liquids, semisolid foods, or solid foods; or received only other foods including plain water, sugar water, other milk, other liquids, semisolid foods, or solid foods. These data were then coded, and the breastfeeding status was classified. Classification was based on the current WHO recommendations (7)—ie, exclusive breastfeeding (breast milk only), predominant breastfeeding (breast milk plus other liquids such as water, tea, or juice), and partial breastfeeding (other food or milk in addition to breast milk). For the purpose of the current analysis, questionnaire data collected during the time of the deuterium study were extracted from the main database, and infants were classified by feeding pattern.

Measurement of breast milk intake

Intakes of breast milk and nonbreast-milk water were estimated 3 mo after delivery by using the dose-to-mother deuterium dilution technique. In addition to quantifying intakes, this method allows for an estimation of the exclusivity of breastfeeding, because the breast milk and nonbreast-milk water intakes can be compared. At baseline, a 2-mL sample of saliva was collected from each mother and a 2-mL sample of urine was collected from her infant, after which the mother received a 10-g oral dose of deuterium oxide (99.8% sterility-tested; CK Gas Products Ltd, Hook, United Kingdom). Another 3 samples of saliva were collected from each mother on days 1, 4, and 14, and another 5 samples of urine were collected from each infant on days 1, 3, 4, 13, and 14. After the analysis of an initial batch of the samples, it was evident that rates of water turnover in these women were high, which necessitated an adjustment in the protocol to include additional saliva samples from the participating mothers on days 3 and 13. Maternal saliva was collected ≥30 min after consumption of any food or drink. The mother was asked to chew on a cottonwool ball for ≈5 min, and saliva was then expressed from the cottonwool by using a plastic syringe. Urine was collected from infants by placing cottonwool balls within a disposable diaper and waiting for the infant to pass urine. The diaper was checked every 15 min, and, if the diaper was dry, clean cottonwool balls were inserted (8). If the infant had passed urine, the cottonwool balls were removed with forceps, and the urine was expressed with the use of a plastic syringe. Urine and saliva samples were then stored at −20 °C before transportation to Cambridge, United Kingdom, for analysis.

The ^2H enrichment in the saliva and urine samples was measured by isotope ratio mass spectrometry at the Elsie Widdowson Laboratory, MRC Human Nutrition Research (Cambridge, United Kingdom). Equilibration with hydrogen gas was performed as described elsewhere (9), with the following slight modifications: a 222 XL liquid handler (Gilson, Middleton, WI) was used to fill sample bottles containing 0.4 mL sample with hydrogen gas by using a venting needle that provides 3 mL hydrogen gas at 1 bar to each bottle. Samples were equilibrated in the presence of platinum catalyst rods online at 22 ± 0.1 °C for 6 h before analysis. The precision of the $^2H/^1H$ measurements was 0.26 ppm.

The intakes of breast milk and of water from nonbreast-milk sources were calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in the mothers and infants and measuring the transfer of milk from mother to the infant, as described elsewhere (10).

Statistical analysis

Comparisons among group means were made by using 2-sample *t* tests. Comparisons between breast milk or nonbreast-milk water intakes and infant and maternal characteristics were tested by using analysis of variance (ANOVA). To validate reported feeding practices against intakes measured by deuterium dilution, 2 methods were employed—ie, the Markov Chain Monte Carlo (MCMC) method and a finite-mixture model (11).

MCMC models were used to estimate the precision with which the deuterium method estimates the proportion of each child's fluid intake that comes from breast milk. This Bayesian technique has the capacity to model complex systems—in this case, the nonlinear simultaneous equations representing the trajectories of the mother's and the child's deuterium enrichment over the 14 d after dosing. The MCMC method's output is a series of random values for each of the model parameters, sampled with probability determined by their posterior distribution (ie, by their "plausibility" as determined by the model and the data to which it is fitted). In our models, the dynamics were represented by a 4-parameter model: 1 parameter each for the proportion of mother's and baby's total body fluids that was replaced per unit of time, 1 parameter for the proportion of the baby's fluid intake that came from the mother's breast milk, and 1 parameter for the mother's initial (postdose) deuterium enrichment above baseline. This parameterization removes the complications—and additional variation—resulting from the need to estimate absolute volumes of fluid and yields a slightly simpler solution to the differential equations than that described by Coward et al (12). Each parameter was fitted as a random effect (following a log-normal distribution whose parameters were given flat, noninformative prior distribution). The MCMC method was used to draw 20 000 samples (after 20 000 "burn-in" cycles to avoid dependence on the initial values; 3 chains were followed to check convergence) from which an estimate of the means (and 95% CIs) of the proportion of intake from breast milk was calculated for each child. As in Coward et al (12), an adjustment was made to allow for water exchange at the lungs. This was achieved by multiplying the proportion of the intake from breast milk by an appropriate factor, calculated to be 1.16. As with all methods, the estimates obtained depend on the validity of the model's assumptions, and it remains uncertain how sensitive the estimates are to model misspecification (eg, to the assumption that the parameters are constant with respect to time). We note that, in 3 children, little or no deuterium enrichment could be detected, presumably because they were receiving little or no breast milk, and consequently their data could not be properly modeled.

In an attempt to estimate the accuracy of the reporting of practices in each feeding and counseling group, a finite-mixture model was applied. This model has been used in many fields to represent mixtures of groups of data that follow normal or other distributions (11). For this model, we assumed that the combined distribution of infant intakes from both the reported EBF group and the reported NEBF group was composed of a mixture of 2 normal distributions, as given in the following equation (11):

$$f(x) = \pi N(\mu_1, \sigma_1) + (1 - \pi) N(\mu_2, \sigma_2) \qquad (1)$$

where $f(x)$ is the overall distribution, N refers to the normal (ie, Gaussian) distribution, μ_1 and μ_2 are means, σ_1 and σ_2 are SDs, and π is the proportion of the total sample that is in the first distribution. The first distribution represents infants with no nonbreast-milk water intake, and the second distribution represents infants with some nonbreast-milk water intake.

A maximum likelihood procedure implemented in the NORMIX software program (version 2.2; proprietary to JH Wolfe) (13) was used to estimate the parameters. Maximum likelihood procedures work by estimating the values of parameters that were most likely to have generated the data in hand, given the model assumed. The parameter estimates were then used to further estimate sensitivity, specificity, and positive and negative predictive values (14). Sensitivity and specificity are measures of the accuracy of the mothers' reports in comparison with the estimation obtained from the deuterium dilution technique. Positive and negative predicted values are measures of the efficiency of the mothers' reports. It should be remembered that this analysis is essentially "illustrative," because the estimates depend on rather strong and unsubstantiated assumptions about normal finite mixtures.

RESULTS

Study population

A total of 101 mother-infant pairs were recruited from the main MINIMat study into the current substudy. Of these 101 pairs, complete data were available for 98, and the remaining 3 pairs were rejected on the basis of incomplete saliva and urine collection. Forty-four of the women had been randomly assigned to the intervention arm of the study, and 54 were from the control group. Mother and infant characteristics according to intervention group are detailed in **Table 1**. Significant differences were observed in parity and in years of schooling, with a higher parity and lower number of years of schooling in the group that received exclusive breastfeeding counseling. No other differences were observed in either maternal or infant characteristics according to intervention group.

Classification of infant feeding practices—reported data

Data on infant feeding practices were coded by using the questionnaire data. Of the subjects, 75 reported that they were exclusively breastfeeding their infant, 6 were predominantly

TABLE 1
Maternal and infant characteristics by randomization group[1]

	Intervention group (n = 44)	Control group (n = 54)
Maternal characteristics		
Age (y)	28.1 ± 5.19 (18.0–39.1)[2]	26.9 ± 5.62 (18.2–43.0)
Weight (kg)	45.0 ± 4.42 (36.5–54.6)	44.7 ± 7.26 (30.5–64.4)
Height (cm)	147.5 ± 4.29 (137.6–156.0)	147.6 ± 5.32 (135.0–162.0)
BMI (kg/m^2)	20.7 ± 1.66 (17.8–26.0)	20.44 ± 2.61 (15.7–28.8)
Parity	2.07 ± 1.66 (0–7)	1.13[3] ± 1.41 (0–7)
Schooling (y)	3.86 ± 3.75 (0–12)	5.67[3] ± 3.81 (0–12)
Wealth index[4]	2.66 ± 1.46 (1–5)	3.19 ± 1.39 (1–5)
Infant characteristics		
Males/females	22/22	30/24
Age (wk)	14.35 ± 1.35 (10.9–16.5)	14.18 ± 1.48 (10.8–17.2)
Birth weight (g)	2677 ± 384 (1480–3403)	2777 ± 387 (1660–3553)
Gestational age (wk)	39.08 ± 2.08 (30.1–42.6)	39.3 ± 1.62 (35.1–43.1)
Weight at follow-up (kg)	5.50 ± 0.81 (3.60–6.90)	5.73 ± 0.70 (3.3–7.4)

[1] The intervention group received the usual health care message plus exclusive breastfeeding counseling; the control group received the usual health care message only.
[2] $\bar{x}$ ± SD; range in parentheses (all such values).
[3] Significantly different from intervention group, $P \leq 0.05$ (2-sample t test).
[4] Wealth index was based on information on household assets and estimated by principal component analysis, producing a weighted score. Scores were grouped into quintiles.

breastfeeding, 15 were partially breastfeeding, and 2 were giving only replacement feeds to their infants. The proportion of women exclusively breastfeeding at follow-up was 79.5% in the intervention group and 74% in the control group (P = 0.5250). The full analysis of the effect of the intervention on the duration of exclusive breastfeeding will be presented elsewhere.

Breast milk intake

Two infants were not breastfed during the study period, and the data from 2 additional infants were excluded from the analysis because of a poor fit in the model used for estimating intakes. Data on breast milk and nonbreast-milk intakes are therefore available for 94 mother-infant pairs. Mean breast milk intake measured by the dose-to-mother deuterium dilution technique was 863.0 ± 170.3 mL/d. No significant correlations were observed between breast milk or nonbreast-milk water intake and any of the infant or maternal variables measured (data not shown). Breast milk and nonbreast-milk water intakes according to reported feeding status are detailed in **Table 2**. A significant difference was observed in both breast-milk (P = 0.0267) and nonbreast-milk water ($P \leq 0.0001$) intakes between the group reported to be exclusively breastfed (reported EBF) and the other 2 groups (ie, predominantly and partially breastfed) combined. However, no significant difference was observed in total water intakes between the groups (P = 0.1929). For all further analyses, the predominantly and partially breastfed groups have been combined to form one group of reported nonexclusively breastfed infants (reported NEBF group; n = 21). No significant differences existed between the intervention and control groups in the intake of either breast milk (866 and 860 mL/d, respectively; P = 0.8535), nonmilk water (70.6 and 66.0 mL/d, respectively; P = 0.8683), or total water (825 and 815 mL/d, respectively; P = 0.7235).

TABLE 2
Breast milk, nonbreast-milk water, and total water intakes by reported feeding group[1]

	Breast milk	Nonbreast-milk water	Total water[2]
		mL/d	
EBF (n = 73)	883.7 ± 163.0 (527–1236)[3]	40.0 ± 80.6 (−99–270)	809.7 ± 120.7 (500–1106)
PredBF (n = 6)	821.2 ± 163.0 (596–975)	119.3 ± 264.6 (−50–639)	834.6 ± 208.3 (542–1158)
PartBF (n = 15)	778.6 ± 189.7 (514–1096)	184.9 ± 198.0 (−71–593)	863.1 ± 191.5 (536–1284)
All infants (n = 94)[4]	863.0 ± 170.3 (514–1236)	68.2 ± 133.0 (−99–639)	819.8 ± 139.8 (500–1284)

[1] EBF, exclusively breastfed (breast milk only); PredBF, predominantly breastfed (breast milk plus other liquids such as water, tea, or juice); PartBF, partially breastfed (food or milk in addition to breast milk). Infant feeding status as recorded by questionnaire date. When PredBF and PartBF groups were combined and compared with the reported EBF group (linear model analysis), significant differences were observed in intakes of breast milk (P = 0.0267) and nonbreast-milk water ($P \leq 0.0001$) but not in total water (P = 0.4549). For individual group comparisons, this difference reached significance only for nonbreast-milk water intakes between the EBF and PartBF groups ($P \leq 0.001$) (Scheffe's post hoc test). No other group differences were significant.
[2] Total water content calculated as water from breast milk (corresponding to ≈81.7% of breast milk intake after adjustment for milk solids) plus other water.
[3] $\bar{x}$ ± SD; range in parentheses (all such values).
[4] Two subjects were not breastfed during the study period, and the data from another 2 infants were excluded from the analysis because of poor fit of the model.

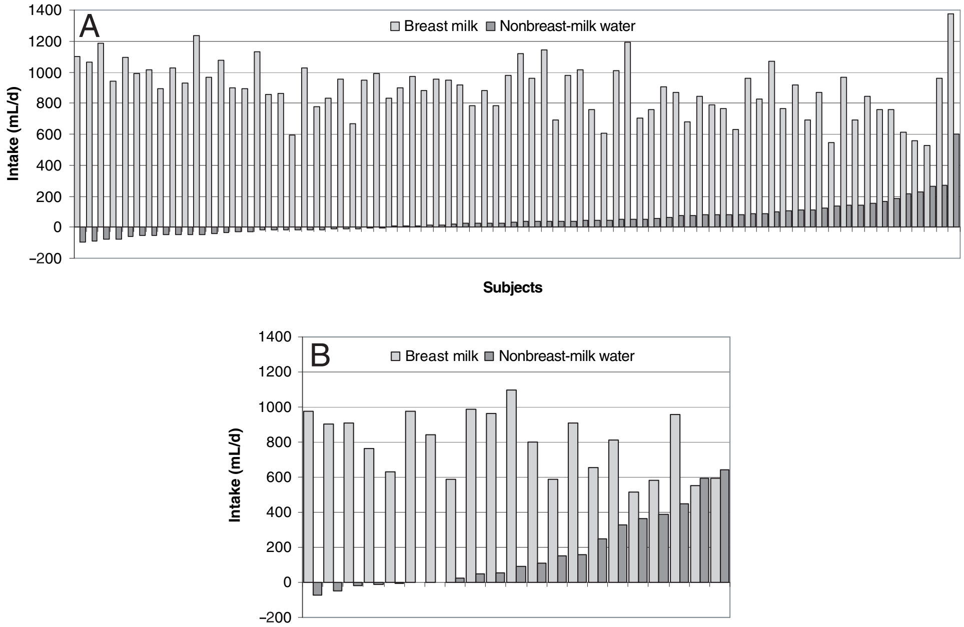

FIGURE 1. Breast-milk and nonbreast-milk water intakes in infants reported to be exclusively (n = 73; A) or not exclusively (n = 21; B) breastfed.

Validation of reported feeding using deuterium dilution technique

Intakes of breast milk and nonbreast-milk water were compared with mothers' reported breastfeeding status. Breast milk and nonbreast-milk water intakes in the infants from the reported EBF group are shown in **Figure 1**A, and intakes in the reported NEBF group are shown in Figure 1B. As illustrated, several negative values were obtained for intakes of nonbreast-milk water. These negative values arise because, in infants with no water intake from nonbreast-milk sources, random methodologic error will produce both negative and positive estimates of nonbreast-milk water intake around a zero mean. Such a negative error has been reported in previous studies using the deuterium dilution method, but no previous study has attempted to estimate this error to validate the mothers' reported practices. We have therefore used MCMC and a finite-mixture model to estimate this error within the current study.

Using the data obtained from the MCMC simulation, **Figure 2** describes the estimated error according to each mother's reported feeding: estimates are expressed as a proportion of the total water intake from breast milk (a value of 1 represented exclusive breastfeeding). As shown, a proportion of the infants from the reported EBF group had intakes of nonbreast-milk water >0, as estimated by error bars that do not cross 1. However, in the reported NEBF group, there also were several infants receiving only negligible amounts of nonbreast-milk water,

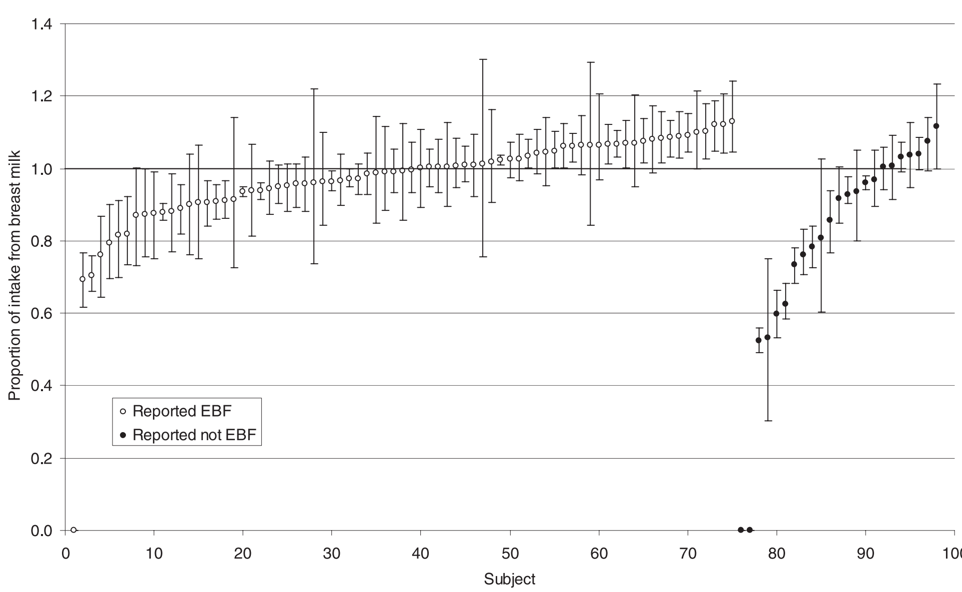

FIGURE 2. Markov Chain Monte Carlo model of total water intake in infants reported to be exclusively breastfed (EBF) or not exclusively breastfed.

TABLE 3
Estimated nonbreast-milk water intake by reported feeding status[1]

	Distribution	Value	Proportion	Count
		mL/d	*%*	*n*
EBF group	1	24.6 ± 62.1[2]	87.4	65
(n = 73)	2	206.7 ± 164.9	12.6	9
NEBF group	1	26.0 ± 64.1	60.6	13
(n = 21)	2	382.2 ± 166.6	39.4	8

[1] EBF, reported to be exclusively breastfed (breast milk only); NEBF, reported to be predominantly or partially breastfed (any foods or liquids in addition to breast milk). Infant feeding status as recorded by questionnaire data. Distribution 1 represents infants receiving no nonbreast-milk water, and distribution 2 represents infants receiving some nonbreast-milk water. Values estimated by using the finite-mixtures model. A likelihood ratio test indicated that a mixture of 2 normal distributions fitted the data better than did 1 normal distribution for both the EBF group ($P < 0.001$) and the NEBF group ($P = 0.03$).

[2] $\bar{x} \pm$ SD (all such values).

which suggests that these infants also may be exclusively breastfed. It should be noted, however, that the figure clearly describes 2 populations, with a greater proportion of infants from the reported EBF group having estimated zero intakes of nonbreast-milk water. We also note that the precision with which the proportion of intake from breast milk is estimated varies considerably. Whereas this proportion typically could be measured with 95% confidence to a precision of ≈7%, the error occasionally (≈8% of babies) could be more than twice that. There appeared to be little correlation between the estimated proportion and its precision, and few large discrepancies between self-assessment and deuterium estimate could be accounted for by especially large imprecision in the deuterium method. The deuterium enrichment of saliva samples from 3 children was insufficient to allow appropriate modeling. They are plotted on the *x*-axis because they are believed to have received little or no breast milk.

To estimate the proportion of misreporting within each group, a finite-mixture model was fitted by using a likelihood method, in which a likelihood ratio test indicated that a mixture of 2 normal distributions fitted the nonbreast-milk water intake data better than did 1 normal distribution for both the reported EBF ($P < 0.001$) and the reported NEBF ($P = 0.03$) groups. The means (±SDs) and proportions estimated from the finite-mixture models are shown in **Table 3**. For both the reported EBF and reported NEBF groups, 1 of the 2 distributions was estimated to have a mean near zero and an SD of ≈60 mL/d. For example, 87.4% of the reported EBF group was estimated to be in the first distribution and to have a mean nonbreast-milk water intake of 24.6 mL/d—a very small amount. Under the assumption that the first distribution represents those with truly no nonbreast-milk water intake, the SD of the first distribution represented measurement error in the estimates of nonbreast-milk water intake. Each of the second distributions had means >0 and larger SDs than those for the first distributions, which supports the assumption that the second distribution group represents the infants receiving measurable intakes of nonbreast milk—thus, those who were not exclusively breastfed. The mean for the second distribution in the reported NEBF group (382.2 mL/d) was larger than that for the second distribution in the reported EBF group (206.7 mL/d), which is consistent with the expectation that the reported NEBF group was consuming nonbreast-milk water. For both the reported EBF and reported NEBF groups, the true variability in nonbreast-milk water intake for the second distribution was estimated to have an SD of 153, obtained by subtracting the measurement error variance estimated from the first distribution from the observed variance of the second distribution and then taking the square root.

The sensitivity of the reporting—ie, the proportion of infants receiving no nonbreast-milk water intakes who were reported to be EBF—was estimated as 0.84 [64.68/(64.68 + 12.73) = 0.84], whereas the specificity—ie, the proportion of infants receiving some nonbreast-milk intake who were reported to be NEBF—was estimated as 0.47 [8.27/(8.27 + 9.32) = 0.47]. The positive predicted value—ie, the proportion reported to be EBF who had no intake of nonbreast milk—was 0.87. The negative predicted value—ie, the proportion reported to be NEBF who had some nonbreast-milk intake—was 0.39.

DISCUSSION

Community-based peer counseling is considered an effective strategy for improving infant feeding practices, especially in populations whose access to health care facilities is poor. Several published studies from a variety of population settings have now reported a significant positive effect of counseling in improving both the rate and duration of exclusive breastfeeding (6, 15–17), and a randomized controlled trial in urban Bangladesh was shown to increase the self-reported prevalence of EBF at age 5 mo from 6% in the control group to 70% in the counseled group (5). Of key relevance in relation to these observations is a study from Dhaka, Bangladesh, that found significantly greater risks of mortality from all causes and specifically from respiratory infections and diarrhea (2.23-, 2.40-, and 3.94-fold risks, respectively) in partially breastfed or nonbreastfed groups than in infants who were exclusively breastfed (18). Reported rates of exclusive breastfeeding in this study were 6% at enrollment (assessed shortly after birth and therefore low because of the use of prelacteal feeds within this population), increased to 53% at 1 mo, and then declined as low as 5% at 6 mo of age. The potential effect on infant health and survival of increasing both the rates and duration of exclusive breastfeeding bears out the need for community-based strategies in both urban and rural settings. However, assessing the true effect of such interventions on infant feeding practices and health outcomes requires a reliable tool for validating the data obtained.

The use of stable-isotope techniques to quantify breast milk intake has several important advantages over previously used methods, such as test weighing and feeding frequency assessment, but the key benefit is that isotopic methods do not interfere with normal routines and behaviors and are not a burden to participating mothers. In addition, measurement of breast milk intake can be performed in the absence of direct supervision of the research team, which encourages normal feeding behaviors. The measurement of breast milk intake by isotopic methods has been compared with test weighing methods in numerous studies, such as the study by Butte et al (19), and a good correlation was observed. In addition, the validity of the method for the assessment of nonbreast-milk water intake has been investigated: a good agreement was observed between the mean nonbreast-milk water intake from weighed bottles and that obtained by using labeled bottles (20). However, this method has not previously been used to objectively cross-validate reported feeding practices in a field-based setting.

A mean breast milk intake of 863 mL/d was observed for all infants participating in the current study: intakes decreased from 884 mL/d in the exclusively breastfed group to 779 mL/d in the partially breastfed group. The mean total intake across the group is comparable to that in other studies in infants from Brazil (10) that used the same isotopic technique. However, to the best of our knowledge, this is the first reported study to use this method to quantify breast milk intakes in Bangladeshi infants. Previous studies from Bangladesh have used the test weighing method to estimate breast milk intakes; reported mean intakes were 632 g/d in children aged 6–12 mo (21), and significant variations were observed according to the month of the year in which test weighing took place (22). The higher intakes observed in the current study than in the study by Brown et al (21) may reflect the fact that infants in that study had a higher mean age than did those in the current study; the higher intakes also may reflect the fact that the infants in the current study consumed a higher total proportion of breast milk or, most likely, may reflect differences between the test weighing used by Brown et al and the isotopic technique used in the current study. That is, test weighing is likely to produce underestimates of intake owing to the artificial situation it imposes on mothers and their infants.

Within the current study, mean intakes of both breast milk and nonbreast-milk water differed significantly between the groups classified on the basis of report as exclusively or nonexclusively breastfed, an observation that provides an initial validation of both the use of the deuterium dilution technique to quantify intakes within this population and also the accuracy in the classification of feeding practices by the mother. It should be noted that, when total water intakes were compared, no significant difference was observed, which suggests a consistent volumetric intake between the exclusively breastfed and mixed-fed infants. Breast milk intakes did not differ significantly between the intervention group and the control group of infants, which suggests that the intervention had no effect on breast milk intakes during the time of this substudy or within the subset of infants included in this substudy. This finding is consistent with an earlier study from Brazil, in which lactation counseling was shown to increase breastfeeding duration but to have no significant effect on breast-milk intake (6).

In an attempt to validate reported feeding practices within the MINIMat study, we have explored the relation between reported feeding behaviors and intakes of breast milk and nonbreast-milk water measured with the deuterium dilution technique. Using an MCMC model to estimate errors within the model, we observed a wide variation in the proportion of total water intake from breast milk in both groups of infants. However, Figure 2 clearly shows that this method discriminates between the 2 groups of infants, with the group reported as not exclusively breastfed having greater intakes than did the EBF group from nonbreast-milk water sources. The MCMC results indicate that the proportion of intake from breast milk can typically be estimated to within 7% of the true value with 95% confidence. Although the precision varies greatly, occasional, very imprecise estimates do not appear to be a major reason for large discrepancies between its estimates and the mothers' self-assessment reports.

To quantify the accuracy of reported practices, we fitted a finite-mixtures model of the volume of nonbreast milk intake separately for the women reporting exclusive breastfeeding and those reporting some nonbreast milk intake. This method depends on the strong assumptions that the subjects can be divided into 2 discrete populations within which the nonbreast milk intake estimates are distributed normally. Whereas these assumptions may be contentious, the results are at least illustrative of the possible scale of misclassification. The method, as implemented here, does not fix one group to a mean of zero (ie, to explicitly define a population of exclusive breastfeeders), but, reassuringly, the estimated means of the lower population in both reporting groups were similar and within 0.33 SD of zero. With regard to the accuracy of the mothers' reported practices, both the sensitivity and the positive predicted values were reasonably high, but the specificity and negative predicted values were not. These values illustrate that, whereas mothers' reports of exclusive breastfeeding accurately and efficiently predicted intake of no nonbreast milk, their reports of nonexclusive breastfeeding were much less accurate and efficient in predicting the intake of some nonbreast milk.

Within the study protocol, women who reported having given *any* nonbreast-milk foods during the study period were classified as not exclusively breastfeeding their infant, although such intakes were not quantified, and thus admitting to having given even very small quantities of nonbreast-milk sources would result in the infant's automatically being categorized as not exclusively breastfed, in accordance with the WHO classification. Thus, one possible explanation why the finite mixtures model estimates a very high proportion (60%) of misreporting among women in the NEBF group is that most women in the NEBF group gave only small amounts of nonbreast milk food or liquid—ie, that the distribution of intakes is not normal, as assumed, but is skewed, with a large proportion of the women in the NEBF group giving a small quantity of nonbreast-milk food. It is almost impossible to determine the relative extent of misreporting and skewing of the distribution; estimates of the proportion of misreported and hypothetical parameters that determine the shape of the distribution would be highly confounded. This apparent overestimation of misreporting in women from the reported NEBF group highlights the possibility that the deuterium dilution technique, although appropriate for quantifying intakes of breast milk and nonbreast-milk water sources during the study period, may not be specific enough to detect very small quantities of nonbreast-milk sources. To improve the precision of the estimates, future studies may consider increasing the number of samples fitted.

In summary, we have shown that the dose-to-mother deuterium dilution technique can be used to measure breast milk and nonbreast-milk water intakes in populations living in rural Bangladesh. This method has several clear advantages over previously used methods. In addition, we describe how this method can be used to validate reported infant feeding behaviors. Within this study, an objective cross-validation between reported feeding practices and measured intakes of breast milk found a high degree of accuracy in the former. No quantitative recommendations on the proportion of water intake that comes from breast milk are associated with the WHO categorization of breastfeeding practices into exclusive, predominant, and partial breastfeeding (1), but it is this value (± measurement error) that the deuterium dilution technique uniquely provides. The present work suggests, however, that the estimated error in measurements, although small enough to allow discrimination between the exclusive and partial categories, is not adequate to distinguish between exclusive and predominant categories in individual infants. With respect to the use of the reported feeding status data for evaluation of the main intervention, whereas this study does highlight some apparent errors in the reporting of infant feeding behaviors, further comparison with other variables, such as the available data on infant growth and morbidity, will act as an internal validation of the main effect of counseling.

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