Original article

Breast milk intake measured by deuterium kinetics in mother-infant pairs in Addis Ababa

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Abstract: The accurate determination of breast-milk intake of infants is essential in order to estimate energy intake and nutrient requirement during infancy and lactation. The deuterium dilution technique was employed for measuring breast-milk intake in exclusively breast-fed Ethiopian infants. This method is convenient for field conditions rather than the commonly used test-weighing procedure. In addition, the feasibility of using the less specialised, more efficient and considerably cheaper instrument, Fourier Transform Infrared (FTIR), was evaluated in the Ethiopian setting. The results obtained were compared to that of Isotope Ratio Mass Spectrometer (IRMS). Ten mother-infant pairs were recruited from two government subsidised health centres, namely Ledeta and Semen. Mothers received a pre-weighed 30g oral dose of D_2O. Maternal and infant saliva samples, and breast milk samples were collected over a 14-day period following dose administration. Anthropometric data were also collected. Saliva and deffated milk samples were analysed for deuterium enrichment by Infrared Spectroscopy and Mass Spectrometry and the data were fitted into two-compartment model. Infant weights were compared with a 12 months breastfed infant-pooled data set. Comparison of these infants with 12 months breast-fed pooled data set showed that weight for age Z-scores were below the mean. There was no significant difference between initial and final Z-scores ($p>0.05$) during the experimental period although all of the infants showed some catch-up growth. Mean ± SD breast milk intake was 850±120ml/day and 880±120ml/day measured using FTIR and IRMS, respectively. The study has demonstrated that it is feasible to measure breast milk intake using deuterium dilution technique in the Ethiopian setting and Infrared spectroscopy could be used for the purpose. It also confirmed that Ethiopian mothers have comparable or higher milk output than privileged communities. These findings have important implications for future research. [*Ethiopia. J. Health Dev.* 1999; 13(3): 271-279]

Introduction

In recent years, attention has been given to the length of time for which breast-milk alone is adequate to support normal infant growth. In most cases the age at which supplementary food should be given is the age at which breast-milk production falls below the requirement. The amount of milk transferred to the infant affects the infant's energy intake and the mother’s energy requirement. The energy requirement of an individual is the level of energy intake from food that will balance

energy expenditure when the individual has body size, composition, and level of physical activity consistent with long term good health (1). For exclusively breast-fed infants, energy requirement is solely based on energy intakes from maternal milk.

The accurate estimation of breast-milk intake of infants is essential to the estimation of nutrient requirements during infancy and lactation (2). The most widely used method for measuring milk intake is the test-weighing procedure in which the infant is weighed before and after each feeding.

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technically simple, requires minimal equipment and training of personnel but prone to measurement

In this method milk intake is usually underestimated by approximately 1% to 5% (3). The method is ____

errors (4). Another method is the mechanical extraction of breast-milk, which is also prone to measurement error (5). It is especially difficult to use the test weighing method in Ethiopia as it requires full co-operation and knowledge of the mother for taking accurate measurements which complicates the matter as most mothers in Ethiopia can not read and write and, as a result, leads to omitting feeds. Therefore, the method of choice is the deuterium dilution technique as test-weighing method is not suitable for field conditions.

The measurement of breast-milk intake by use of the deuterium dilution technique was first introduced by Coward et.al. (6). In the original method deuterium oxide is administered to the infant. This resulted in overestimation of the infant total body water and also unreported water intake from other sources other than human milk (6,7). An improved version of this method, where the dose of deuterium is given to the mother, avoids these problems.

The isotope dilution method does not interfere with feeding behaviour and is therefore suitable for longitudinal studies in developing countries (9).

In this study Infrared Spectroscopy was used for measurement of breast-milk intake and the results were validated against the gold standard Mass Spectrometric measurement. The application of this non-invasive, convenient method is tested in the Ethiopian setting. This study was done as part of a large-scale study designed to measure the breast-milk intake of exclusively breast-fed infants in Addis Ababa, Ethiopia.

Methods

Ten women of low socio-economic status were recruited from two government subsidised health centres, namely Semen and Ledeta Health Centers, immediately after the birth of the infant. All infants were full-term and the appropriate size for gestational age. At the time of the study all infants were aged less than four months, exclusively breast-fed, and free from infection. Ethical approval for the study was obtained from the Ethiopian Health and Nutrition Research Institute's Ethical Committee. Written informed consent was obtained from all participating mothers.

On study day "0", a pre-weighed 30g dose of neat deuterium oxide was administered orally to each mother irrespective of body weight, following the collection of the pre-dose samples and anthropometric data. This was in agreement with an earlier study by Caballero(10). The use of a fixed isotope dose simplified the procedure, bypassing the need to know each subjects weight before preparing individual doses, and is acceptable provided an accurate record of each mother's weight is made on day "0". Samples of breast-milk, maternal and infant saliva were collected over a 15 day period, (day'o' through day '14'), commencing with a pre-dose sample on day 'o' to determine background enrichment, followed by sampling, on days 1,2,3,4,13 and 14 after dose administration.

Breast-milk samples (approximately 10-20ml) were obtained by manual expression. Saliva samples of between 3-5 ml were collected by having the mothers expectorate into clean plastic tubes. To obtain infant saliva, foam mouth swabs were cut down to approximately 1.5cm cubes, and rolled around the infant's mouth until saturated. The foam was then squeezed into 5ml sterlin plastic tubes. At least 30 minutes had to elapse between the last feed and the collection of the sample to avoid contamination by residual breast milk. All samples were placed in a cool bag on collection in the field. Later samples were stored at-70^{o} C.

On day '0' of the study, mothers were weighed to the nearest 100g using a standard clinical scale (TEFAL, sensitive computer, 130kg/100), and their height was measured to the nearest 0.1cm. Mid upper arm circumference was determined and triceps, biceps, sub-scapular, and superailiac skinfold thickness were measured in triplicate using Lange Callipers (Holtazan Ltd, Cry Mych, UK). Infants were weighed naked to the nearest 100g using an electronic baby scale (Mod. 727-SECA, CMS Weighing equipment Ltd). Length was measured using a portable length board (Pedobaby, Brussels, Belgium).

These measurements were repeated on the last day of the study. The growth of these infants was compared with the new international growth reference prepared by WHO based on children who are fed according to WHO recommendations, which entail exclusive breast-feeding for the first 4-6 months of life. Infant weights were plotted on growth charts derived from pooled 12-month growth data for breast-fed children (11). Weight-for-age Z-scores were calculated and a paired t-test applied for significant change in Z score over the experimental period.

Laboratory p*rocedures*: Approximately 1.5ml of breast-milk and maternal saliva were centrifuged at 13000 rpm for five minutes, as required. The deffated milk samples were transferred to clean tubes, as was the supernatant obtained from each saliva sample. The whole sample was centrifuged at 13000 rpm for five minutes. The sample was recovered after FTIR analysis to be reused in later analysis by isotope Ratio Mass Spectro-meter.

The Fourier Transform Infrared (FTIR) Spectrometer was used to determine the ^{2}H enrichment of saliva and breast-milk samples. This method is based on the detection of the vibrational band produced by deuterated water between 2400 and 2600cm^{-1},in the infrared region of the spectrum. Pre-dose samples were used as a reference against which the corresponding post-dose samples were read. These measurements were made in duplicate, while measurements of the standard against distilled water were made in triplicate at least three times a day in order to calibrate the instrument. Measurements are expressed in parts/million (ppm) excess that is above background enrichment.

Mass Spectrometric analysis was performed by using a Sira 10 (Vacuum Generators Ltd, Middlewich, Cheshire, UK). The saliva samples were centrifuged prior to equilibration to remove solids, but tests on centrifuged and non-centrifuged breast-milk indicated that the

results were not influenced by centrifugation and so this step was not performed. Samples were measured in duplicate and calibrated by the inclusion of two references of differing isotopic composition in each loading list to allow SMOW/SLAP corrected values to be obtained.

Data analysis and modelling: To estimate the agreement between deuterium enrichment measurements obtained for breast milk with those for maternal saliva, the mean values obtained for milk and saliva were plotted against the difference between the values at each data point, (12). Breast-milk intake was estimated from a two-compartment model, describing the monoexponential decay curve of deuterium in the mother's milk and multi-exponential curve of deuterium appearance in the infant's saliva(9). The model does not include a time delay. A spreadsheet devised in Microsoft Excel was used to calculate the various parameters of water kinetics. Each mother-infant pair was modelled individually using values obtained from the analysis of infant and maternal saliva.

The infants total body water (TBW) was calculated from the equation of Butte et al. (8). Single estimate of body water was considered inappropriate since the infants showed a weight increase over the two-week period during which samples were taken. Estimates were made from the baby's initial and final weights, and intermediate values calculated by linear interpolation. Maternal total body water pool was calculated as the hydrogen distribution volume divided by a factor of 1.04 to account for non-aqueous exchangeable hydrogen. From this the weight of lean tissue was estimated, assuming it to be 73% hydrated, and the amount of fatty tissue calculated as the difference between the mothers total and lean tissue weights.

Statistical analysis: A paired t-test was carried out to discover whether the final z-scores were significantly different from the initial Z-scores. Bland and Altman plot was made to see the agreement between breast-milk and saliva measurements on FTIR(12).

Table 1**: Maternal anthropometric characteristics**

Subject	Age (yrs)	Height (m)	Weight (kg)	BMI (kgm^{-2})	MUAC (cm)	Sum skin-folds (cm)	Predicted body fat (%)
1	20	1.62	48.40	18.47	22.00	54.90	27.79
2	22	1.65	51.80	19.04	23.75	87.20	34.34

3	30	1.77	73.00	23.32	29.70	80.80	34.34
4	28	1.62	61.40	23.44	25.35	59.60	28.72
5	22	1.64	56.00	20.82	26.30	53.60	27.33
6	23	1.54	51.60	21.76	25.00	51.70	26.87
7	26	1.59	62.30	24.60	25.55	76.10	32.45
8	31	1.55	45.50	18.96	23.40	47.13	27.33
9	38	1.51	47.00	20.75	24.50	53.53	29.18
10	22	1.56	51.50	21.19	24.30	52.70	27.33
Mean	26	1.61	54.8	21.2	24.9	61.7	29.57
SD	6	0.07	8.5	2.1	2.0	14.1	2.83

Results

Age, weight, height and skinfold-thickness of the mothers are presented in Table 1. The BMI values for the mother range from 18.5 to 24.6 showing that none of them ware malnourished (13). The mean mid-upper arm circumference 24.99 was 90% of the standard of WHO reference (14), indicating that maternal body fat stores are adequate for breast-feeding. Skinfold measurements were converted to an equivalent fat percentage using the equation developed by Durnin and Womersley (15) to predict body density, from which body fat content can be predicted using Siri's equation (16).

Mean ± SD initial infant weight was 4.46 ± 0.75kg and mean ±SD final weight was 4.99 ± 0.83kg, with an average increment of 0.53 ± 0.28kg. Comparison of these infants with a "12 month breastfed pooled data set"

Table 2: **Infants characteristics**

Subject	Sex	Age (d)	Initial Weight(kg)	Z-Score	Age(d)	Final Weight(kg)	Z-Score	Change Weight(kg)	Z-Score
1	M	25	3.77	0.50	39	4.50	0.00	0.73	0.50
2	F	31	3.85	-1.00	45	3.94	-2.00	0.09	1.00
3	M	41	4.95	1.00	55	5.09	-1.00	0.14	2.00
4	M	31	4.35	-0.50	45	4.95	-1.00	0.60	0.50
5	F	45	4.30	-1.50	59	4.65	-1.00	0.35	-0.50
6	F	63	4.00	-2.00	77	4.50	-2.00	0.50	0.00
7	M	45	5.61	0.00	59	6.40	1.50	0.79	-1.50
8	M	35	3.90	-1.00	49	4.40	-2.00	0.50	1.00
9	F	45	4.00	-2.00	59	4.95	0.00	0.95	-2.00
10	M	101	5.85	-0.50	115	6.50	-0.50	0.65	0.00
Mean		46	4.46	-0.70	60	4.99	-0.80	0.53	0.10
SD		22	0.75	1.01	22	0.84	1.11	0.28	1.20

Table3: **Summary of the data obtained from the Mass Spectrometric and Infra-red methods. In each case the results are quoted as the mean of the ten subjects with the standard deviations following in parentheses.**

	Mass SpecS	FTIR	
MOTHER			
age	26(6)	26(6)	Years
weight	54.9(8.5)	54.9(8.5)	kg
D space	30.4(5.3)	29.8(5.0)	(dm)3
Lean Mass	0.0(6.9)	39.3(6.6)	kg
Body fat	4.9(3.3)	15.6(3.6)	kg
fat%	7.1(4.5)	28.3(4.7)	
wat intake	3.87(0.68)	3.82(0.66)	$(dm)^3$ day^{-1}
BABY			
Age	46(22)	46(22)	days
Start weight	4.5(0.8)	4.5(0.8)	kg
Final weight	5.0(0.8)	5.0(0.8)	kg
Start ext water	-0.03(0.05)	.00(0.07)	kg.day1
final ext water	0.0.8(0.02)	.08(0.01)	$kg.day1^{-1}$
KINETIC			
	k(mm)=0.128(0.013)	0.129(0.015)	day1
	k(bb)= 0.267(0.031)	0.270(0.035)	day1
	k(bm)=0.025(0.003)	0.025(0.004)	day1
	F(bm)=0.76(0.10)	0.74(0.10)	kg.day1
	Milk vol 0.88(0.12)	0.85(0.12)	kg.day1

show that the mean initial Z-score was 0.7 ± 1.01 and the me an final Z-score was 0.10 ± 1.20 (Table2). Eventhough 90% of the infants had weight-for-age Z score below the mean, none of the infants were observed to have weight for age Z-scores below – 2SD. There was no significant difference between initial and final Z-scores ($p>0.05$) although all of the infants had shown some catch-up growth.

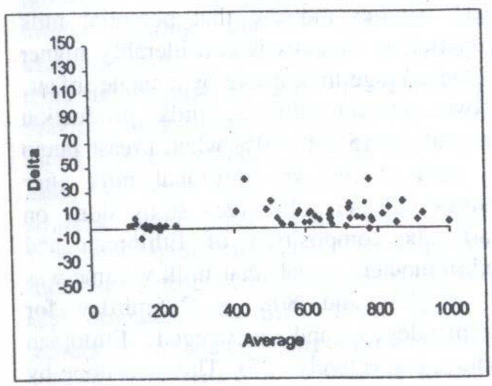

Figure 1: **Bland and Altman analysis of the Mass Spectrometric results obtained from maternal saliva versus breast-milk.**

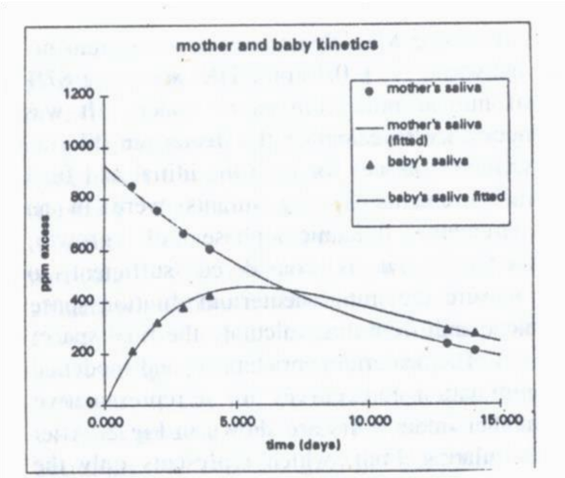

Figure 2: **Modelled elimination curve for a representative mother-infant pair.**

The Bland and Altman plot (Fig.1) for saliva and breast-milk showed that results from the two fluids, breast-milk and maternal saliva, were therefore not significantly different. Without exception, saliva data showed greater precision than that obtained from breast milk.
Table 3 summarises the parameters of water kinetics for the 10 subjects analysed. The mean steady state transport of water to the infant from the mother (Fbm) was 0.760 ±

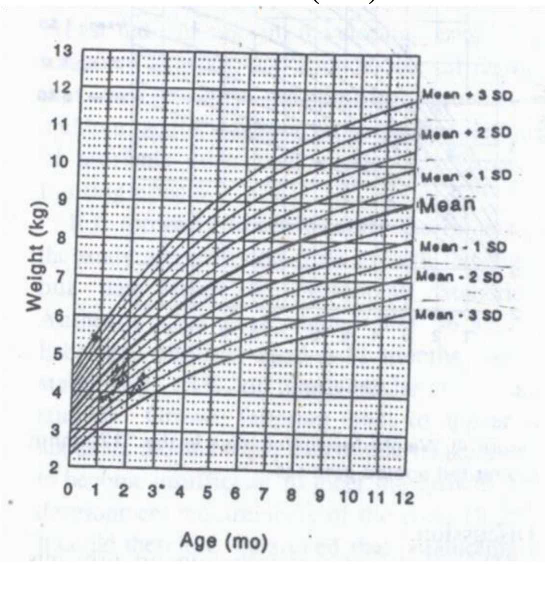

Figure 3: **Weight-for-age of girls in the "12-month breast-feed pooled data set"**

0.10, using Mass Spectrometric measurements and was 0.74 ± 0.10 on FTIR, assuming 87% of human milk consists of water. It was necessary to calculate the deuterium dilution space (D space) for both the initial and final measurements as the infants were in an extremely dynamic phase of growth. Generally, it is considered sufficient to measure the initial deuterium dilution space alone and, from this, calculate the final space, (17). The deuterium enrichments and modelled elimination rate curves for a representative mother-infant pairs are shown in Fig.2. After calculating Fbm, which represents only the free water of milk and does not include the water obtained from the oxidation of milk solids, milk output was calculated as M=Fbm/0.87. Therefore, the average milk output was 880 ml/d using measurements on MS and 850 ml/d using FTIR. There is no significant difference between milk output measured by the two methods.

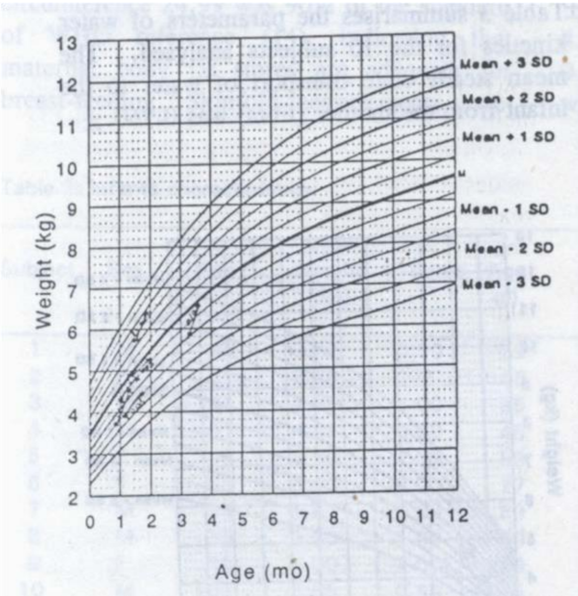

Figure 4: **Weight-for-age of boys in the "12-month breast-fed pooled data set"**

Discussion

The results of the study support previous studies (8,9,17) that it is possible to conduct investigations into breast-milk intake using the deuterium dilution technique in underprivileged populations in developing countries, in this case Ethiopia.

Concerns had been expressed that the fixed dose of 30g $D_2 0$ would not achieve adequate isotopic enrichment in the saliva samples towards the end of the elimination period for detection by the less sensitive Infrared Spectroscopy method. However, not only was the Infrared Spectrometer able to detect enrichment at values of less than 200ppm, the precision as estimated by the coefficient of variation, was well below the accepted limit of 5%. The measurements obtained by FTIR were validated against IRMS and the results for all kinetic parameters and breast-milk output were comparable. Therefore, it is feasible to use Infrared Spectroscopy in the analysis of samples; thus corroborating the study carried out by Caballero et al. (10) in Indonesia.

There is a very wide range in milk intake among healthy, exclusively breast-fed infants. In industrialised countries, milk intakes average approximately 750 to 800ml/d in the first four to five months, but range from approximately 450 to 1,200ml/day (18,19,20,21). Most of these studies used the test weighing method, which tend to underestimate milk production when compared to the deuterium dilution technique. Data from developing countries indicate a similar mean level of intake when a rigorous methodology for measuring milk volume is used (22). Several studies indicate that potential milk production in humans is considerably higher than the average milk intake by a single infant. In two separate studies, milk production increased by 15% to 40% when breast pump was used to remove additional milk after feedings (23,24). Previous study done on breast-milk composition of Ethiopian and Swedish mothers reveals that milk volume was 769 ± 354 and 808 ± 225ml/day for underprivileged and privileged Ethiopian mothers, respectively (25). This was done by using a rigorous methodology of emptying both breasts by using an electrical pump. Using the safe and non-invasive method of deuterium dilution, breast milk intakes of the Ethiopian infants in this study were similar to or exceeds those commonly reported for exclusively breast-fed infants in privileged and underprivileged populations (8,26). Human milk intakes (648.0±63.1ml/d) of priveileged breast-fed infants from huston (27), and of 23 Papua New Guinean infants (mean ± sd: 606 ± 100ml/d at one month; 734 ± 164ml/d at two months) receiving minimal supplementation (9), are lower than those estimated using the same D_20 dose-to-mother method as in this study where the milk intake for 10 infants (mean age, 60 days) was 880±120ml/d using Mass Spectrometric method and 850±120ml/day using the Infrared method.

The fact that most of these mothers were from low socio-economic class does not seem to affect milk production. The most important variable behind lactation failure in poor women, namely milk volume, does not not seem to be dependent on food intake, but the combined stress of maternal disease and undernutrition. The mothers in this study were healthy and with adequate fat stores which may have contributed to high milk output.

The dose-to-mother method for measuring breast-milk output is non-invasive simple and safe, and involves minimal disruption to feeding behaviour. In the original deuterium dilution technique developed by Coward (6), the dose was given to the infant and not to the mother. However, this method demands longer fasting periods for the infant immediately preceeding sample collection, and more significantly, is based on the assumption that the only water source presented to the infant is breast-milk, (28,2). If this is not the case, all food and water consumed from sources other than breast milk must be recoreded quantitatively (27). The revised method of dose administration to the mother (28) obviates these difficulties. Milk-water volume is calculated using the compartmental model, from which it is possible to estimate the amount of supplemental water intake (Fbo), thus enhancing the accuracy of estimates for milk intake.

Comparison of these infants with the 12-month breast-fed pooled data set (11) revealed that 90% of these infants were below the mean (Fig 3&4). However,this is not necessarily indicative of undernutrition, but may instead illustrate the non-transferable nature of reference data between populations, particularly when applying figures based on affluent populations to those in deprived areas. Contrary to the high breast-milk intake and considering the mean age for these infants (60days), it can not be concluded that they were not growing very well. Furthermore, the aetiology of infant growth faltering in underprivileged populations is not fully understood (8). It was shown that early growth faltering is not attributable to breast-milk intake. Despite human-milk intakes of 885±145 and 869±150ml/day at four and six months, respectively, growth faltering was evidenced by a significant decline in growth velocities and NCHS scores. Similarly, in this study despite the high breast milk intake, the growth of the infants was below the mean for exclusively breast-fed infants(11). Therefore, growth faultering, is unlikely, however, to be solely due to inadequate lactation (29,30). This suggests that there may be other factors, besides nutritional, for the growth failure in breast-fed infants in developing countries. Infection, genetics, and altitude, are all factors that may modify the physical growth of children, and it is highly probable that deficits of nutrients other than energy have growth limiting effects.

It is too early to see whether the infants in the study are suffering from growth faltering, but data from the Ethiopian Statistical Authority (30) have shown that 56.5% of Ethiopian children aged 611 months, and a staggering 64% of those under five, are stunted. Growth faltering tends to appear at about six months when breast-milk intake starts to become insufficient to meet the growth and development requirements of the child (8,29). It could therefore, be argued that conducting a largescale survey into the breast milk intakes of infants aged less than four months is not targeting the group most at risk. A better use of resources perhaps, would be to determine the intakes of babies

4-12 months of age, and on the basis of this information, formulate public health policies concerned with the optimum time for introduction of weaning foods. There is, however, a huge amount of evidence supporting the adequacy of breast- milk as the sole source of nutrition for at least the first four months of life, (32,33,34). Indeed, it may be that in underprivileged environments where the introduction of supplementary foods and fluids of poor nutritional quality, in unsanitary conditions, is associated with a heightened risk of malnutrition and infection, exclusive breast-feeding should be promoted. To conclude, the study has demonstrated that it is feasible to measure breast milk intake using the deuterium dilution technique in the Ethiopian setting. It also confirmed that lactating Ethiopian mothers have comparable or higher milk output than privileged communities. These findings have important implications for future research.

Acknowledgements

We would like to thank the mothers who participated in the study for their co-operation and hospitality. Our sincere thanks to the International Atomic Energy Agency for funding the project and the EHNRI for logistical support.

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