

MEASUREMENT OF BREAST MILK INTAKE USING DEUTERIUM OXIDE AND FOURIER TRANSFORMED INFRARED SPECTROPHOTOMETER - A PILOT STUDY

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ABSTRACT

The measurement of breast milk intake of infants is essential to the estimation of nutrient requirements during infancy and lactation. The conventional method, test-weighing procedure for measuring breast milk is time consuming, most often inaccurate and may interfere with the mother's normal activities. A more practical and accurate method is isotope dilution using stable isotope-labelled water. The accuracy and ready availability of deuterium oxide (D₂O) have led to its extensive use in measuring body composition and breast milk intake of infants. The D₂O turnover method was field-tested in 13 lactating Ghanaian mother-baby pairs. Maternal and baby anthropometric measurements were made. Baby milk intake and maternal body composition were measured with the dose-to-mother method. Pre-dose samples of saliva were taken from each mother-baby pair. A measured D₂O dose (30g) was administered orally to the mother. Post-dose saliva samples were collected from mother and baby on days 1, 2, 3, 4, 13, and 14. Samples were analysed using Fourier Transformed Infrared Spectrophotometer (FTIR). The mean \pm SD maternal age was 24 ± 5 years. Babies were aged 3.5 months on the average and weighed 6.7 ± 0.7 kg. Mean milk intake of babies was 828 ± 132 ml/day with a range of 610 to 1040 ml/day. Maternal fat free mass and % body fat were 44.8 ± 5.3 kg, 23.1 ± 5.1 respectively. This non-invasive and convenient method has been used successfully to measure breast milk intake of Ghanaian infants.

INTRODUCTION

Measurement of breast milk intake is fundamental for infant nutrition in developing countries. In sub-Saharan Africa, the practice of breastfeeding is common and is the most economically feasible and culturally acceptable method of infant feeding. It is well-documented that early introduction of weaning foods is one of the causes of child malnutrition (Cohen *et al.*, 1994). In spite of the many advantages as-

sociated with exclusive breastfeeding during the early stages of development, the proportion of infants who are exclusively breast-fed in Ghana is small (GDHS, 2003).

The conventional method for measuring breast milk intake is the test-weighing procedure in which the baby is weighed before and after each feeding. This method is time-consuming, most often inaccurate i.e. milk intake is usually underestimated by approximately 1% to 5%

(Brown *et al.*, 1982) and may interfere with the mother's normal activities. A more practical and accurate method is isotope dilution using stable isotope-labelled water. Deuterium oxide (D_2O) is given to either mother or baby, saliva or urine samples collected over a period of time and sample enrichments measured with the isotope ratio mass spectrometer (IRMS). The IRMS is expensive, time-consuming, requires specialist for operation and maintenance, and difficult to set up in developing countries.

A fast, easy and less expensive method using the Fourier Transformed Infrared Spectrophotometer (FTIR) has been developed to measure deuterium enrichment in samples. Coward *et al.* (1979) first introduced the deuterium dilution technique for measurement of breast milk intake which involves administration of the D_2O to the baby. Although it has obvious advantages over the test-weighing, this method demands longer fasting periods for the infant immediately preceding sample collection. Estimation is based on the assumption that the only water source presented to the baby is breast milk (Coward *et al.*, 1979; Butte *et al.*, 1983); water intake from sources other than human milk is not reported. Moreover, infant total body water is significantly overestimated. The method used in this study where D_2O is administered to the mother, is an improved version, which helps reduce these difficulties.

The dose-to-mother D_2O turnover technique allows estimation of mother's body composition and breast milk intake in addition to non-breast milk water intake (Coward *et al.*, 1982; Orr-Ewing *et al.*, 1986; Butte *et al.*, 1988). Few studies have used the D_2O method to investigate milk intake of breast-fed infants in developing countries (Cisse *et al.*, 2002; Etyyang *et al.*, 2004; Etyyang *et al.*, 2005). This study tested the feasibility of D_2O and FTIR in measuring milk intake in breast-fed babies in Ghana.

SUBJECTS AND METHODS

Subject recruitment

Thirteen (13) lactating mother-baby pairs were recruited from Half Assini in Western Region

of Ghana. Selection criteria included, mothers having a baby aged 3-4 months with no congenital abnormalities, babies who were being breast-fed, and mothers who had no intention of travelling outside their communities during the study period. The study was approved by the Ethical Review Committee of the Health Research Unit of the Ghana Health Service. Objectives and procedures of the study were explained to the mothers after which written informed consent was obtained from all participating mothers.

Anthropometry

Mean age of babies was 3.5 months and mothers averaged 24 ± 5 years. Maternal weights were measured to the nearest 0.1 kg using an electronic scale (SECA). Infants were weighed to the nearest 0.1 kg with an infant weighing scale. These were done at the beginning and end of the study. Maternal heights were measured to the nearest 0.1 m using a stadiometer. Recumbent lengths of babies were taken with an infantometer to the nearest 0.1m. Ages of babies were obtained from Child Welfare Cards (weighing cards) and maternal ages by interviews.

Deuterium dilution

Breast milk intake was measured using the D_2O turnover technique (Coward *et al.*, 1982). Pre-dose saliva samples of between 3-5 ml were collected from mothers directly into small sterile vials. Similar volumes of saliva samples were collected from the babies, using cotton balls as mouth swabs. When saturated with saliva, the cotton balls were placed in a syringe and squeezed into sterile tubes until required volume had been collected. A measured D_2O dose (30 g) [99.8% purity, Cambridge Isotope Laboratories Inc, Andover, MA] was administered orally to the mothers. Post-dose saliva samples were collected from both mothers and babies on days 1, 2, 3, 4, 13 and 14. The samples were transferred to the laboratory on ice, centrifuged for 5 minutes at 11,500 g and the supernatant stored at $-20^\circ C$ prior to analysis.

Analysis by FTIR

Enrichment of saliva samples was measured using FTIR (Shimadzu 8300, Vienna, Austria) at the Medical Research Council-Human Nutrition Research (MRC-HNR) Cambridge, United Kingdom. Calibration of the FTIR was done by preparing D₂O standard solution and the enrichment of this calibrator was confirmed by IRMS. Saliva samples were analysed by loading pre- and post- samples into the instrument and positioning in the beam light. The infrared spectra were measured in the range of 2300 to 2800cm⁻¹.

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 for calibration of the FTIR. Measurements are expressed in parts per million (ppm) excess deuterium above background enrichment.

Breast milk intake was estimated from a two-compartmental model, describing the mono-exponential decay curve of deuterium in the mother's milk and multi-exponential curve of deuterium appearance in the baby's saliva. 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-baby pair was modelled individually using values obtained from the analysis of baby and maternal saliva. Breast milk intake was calculated from FTIR spectra using Microsoft Excel software for kinetic analysis. Maternal body composition was calculated from the total body water (TBW) component.

Data analysis

Data entry and statistical analysis were done by SPSS 12.0 for window. Results are expressed as means \pm standard deviation.

RESULTS AND DISCUSSION

The evaluation of breast milk intake is of particular importance in investigating the effect of diet and environmental factors on maternal

capacity for adequate lactation. The isotope dilution method does not interfere with feeding behaviour and is therefore suitable for longitudinal studies in developing countries. In this study, infrared spectrophotometry was used for measurement of breast milk intake and maternal body composition. This application is non-invasive, safe and accurate. The 7-day sampling regime i.e. on days 0, 1, 2, 3, 4, 13 and 14 gives the best estimates of water turnover (Coward *et al.*, 1982). Calculation of human milk intake and intake of water from sources other than human milk is based on a two-compartment, steady-state model. The mother's body water (V_m) is the first compartment and the baby's body water (V_b) is the second compartment. These two compartments are connected by the flow of milk from the mother to the baby (F_{bm}). The model assumes the total water input is equal to the total water output (Coward *et al.*, 1982) as illustrated in Fig 1.

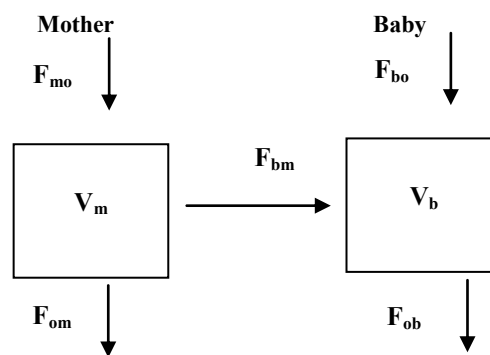


Fig 1: Two compartment, steady-state model of water flows in a mother-baby pair. F indicates water flow. Subscript m refers to mother, o to outside and b to baby. V is the total body water. Example F_{bm} indicates the flow from mother to baby (breast milk intake)

The model was used to generate best-fit estimates for maternal and baby water fluxes. It predicts a mono-exponential decay curve of deuterium in the mother's body water and a bi-exponential decay curve for the appearance of deuterium in the baby's saliva (Figs. 2 and 3).

During the two week study period, the deuterium level gradually decreases in the mother's body water. If the baby is being breast-fed, deuterium enrichment gradually increases in the baby's body water and then declines. A mother who produces more milk has a faster water turnover i.e. faster disappearance of deuterium from the body water. The higher the enrichment in the baby's body water, the higher the volume of milk consumed.

The mother in Fig. 2 was practising exclusive breast feeding. However, in Fig. 3, breast milk is being displaced by intake of other foods or water supplements. Thus whereas mother's deuterium enrichment was decaying, its appearance in the baby's body water was very low throughout the sampling period.

Kinetics data has been presented in Table 1 for one mother-baby pair. Maternal TBW pool was calculated from the deuterium enrichment at time zero, corrected by 1.041 (deuterium space). This is because deuterium (4.1%) ex-

changes with exchangeable hydrogen atoms in body proteins. Deuterium is also sequestered into fats and proteins as these are synthesised. These result in greater dilution space of deuterium than the TBW pool (Racette *et al.*, 1994). Maternal fat free mass (FFM) was calculated as $TBW/0.73$ assuming it is 73% hydrated; body fat (FM) calculated as the difference between the body weight and FFM. Average maternal FFM and percent body fat were 44.8 ± 5.3 kg and 23.1 ± 5.3 respectively.

Table 2 shows characteristics of babies and breast milk intake. The baby's total body water (TBW) was calculated from the equation of Butte *et al.* (1988). Breast milk intake was calculated as $F_{bm}/0.87$, where 0.87 is the fraction of milk that is water, and F_{bm} the flow from the mother to the baby. Other oral intakes of baby other than breast milk (water and food supplements given to the baby) were estimated from F_{bo} , correcting for insensible water loss, i.e. water lost from the body in breath and from the skin by routes other than the sweat glands.

2H Kinetics

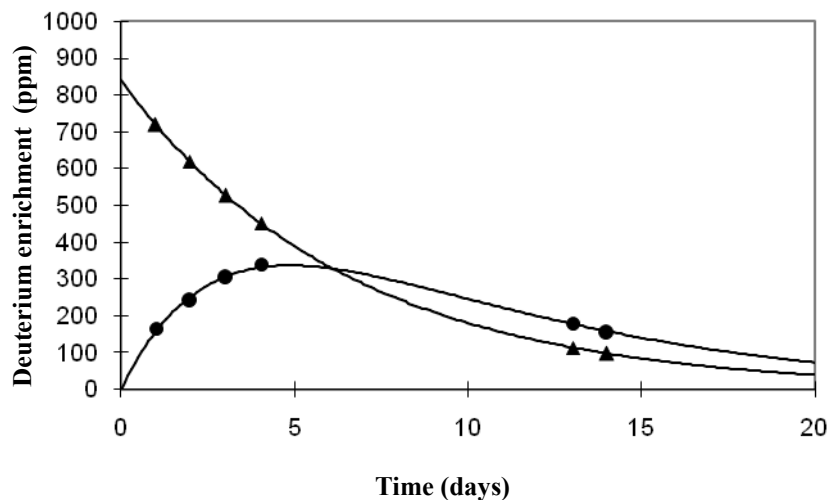
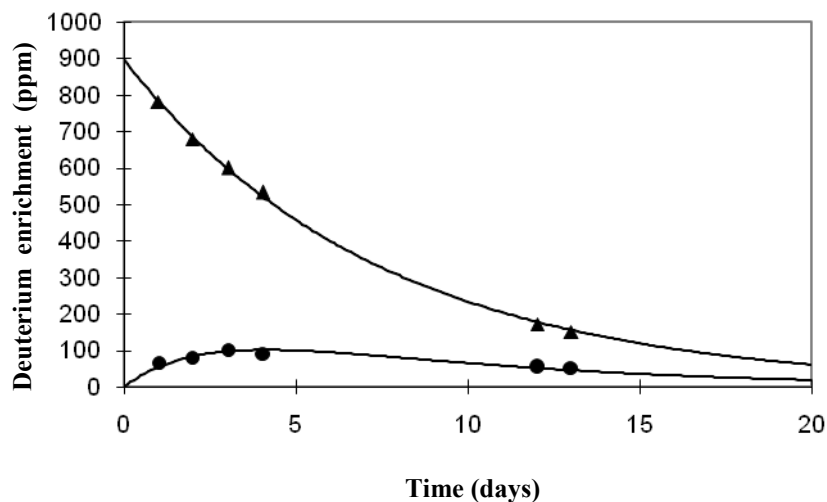


Fig. 2: Decay curves of deuterium enrichment in the body of a mother (▲) and her baby (●) who was exclusively breastfed

2H Kinetics

**Table 1: Kinetics data of one mother-baby pair**

Mother's body composition	Baby's intakes	Kinetic data of a mother-baby pair ¹
Total body water = 37.43 kg	Breast milk intake = 800 g//day	Cm(0) 833.54 ppm
Fat free mass = 47.4 kg	Non oral water intake = 50 g/day	Kmm = 0.23 day ⁻¹
Body fat = 17.05 kg	Non-milk oral intake = 10 g/day	Kbb = 0.22 day ⁻¹
Percent body fat = 26.46 %		Fbm = 0.73 kg/day ⁻¹

¹Cm (0) is the initial deuterium enrichment and kmm, kbb are fractional rate constants

Table 2: Characteristics and breast milk intake of babies

Subject	Gender	Age (mos)	Weight (kg)	Breast milk intake		Non-milk oral intake (ml/day)	Non-oral water intake (ml/day)
				*(ml/day)	(ml/kg)		
PC1	Female	3.2	6.5	870	134	140	54
PC2	Female	3.4	6.6	670	102	420	60
PC3	Male	3.3	6.8	900	132	120	38
PC4	Male	4.1	7.4	1040	141	200	52
PC5	Female	3.0	6.8	950	140	17	50
PC6	Male	2.7	5.3	720	136	230	38
PC7	Male	3.0	6.2	1010	163	19	50
PC8	Male	3.1	7.2	900	125	170	61
PC9	Male	4.0	7.7	820	106	430	33
PC10	Female	4.0	6.5	700	108	260	41
PC11	Female	4.3	6.8	780	115	60	47
PC12	Female	4.2	5.5	610	111	470	46
PC13	Male	3.7	7.2	800	111	50	10

*Mean milk intake 828 ± 132 ml/day

Breast milk intake averaged 828 (132) ml/day, ranging from 610 to 1040 ml/day while other metabolic water intake (food or water supplements) averaged 199 ml/day with a range of 17 to 470 ml/day. Although not significant, male babies tend to consume more milk than females babies (884 ± 115 compared to 763 ± 129 ml/day; $p = 0.10$). A wide range of milk intake among healthy, exclusively breastfed infants have been reported. In industrialized countries, milk intake range from 450 to 1200 ml/day with an average intake of 750 and 800 ml/day in the first four to five months (Hofvander *et al.*, 1982; Dewey and Lonnerdal, 1983). The method used in most of these studies was test weighing which tend to underestimate milk production when compared to the deuterium dilution technique.

The D₂O turnover technique is especially useful for estimating the contribution of breast milk to nutrient intake in non-exclusively breast-fed infants (Coward *et al.*, 1982). The FTIR method is a much cheaper, easy to use and less complicated than the IRMS, which has been used extensively in measuring D₂O enrichment. This method has been validated against IRMS (Jennings *et al.*, 1999; Fusch *et al.*, 1993) and it is most suitable for field conditions particularly in developing countries. Although a higher dose of D₂O is required (30g as opposed to 3g in IRMS), this amount does not pose any risk for the subjects (Coward *et al.*, 1979; Lukaski and Johnson, 1985). The FTIR detected values as low as 30ppm and the mean difference between the theoretical and experimental data was less than the acceptable limit of 5%. Our results indicate that exclusive breast feeding was hardly practiced by these women although they all claimed complementary foods had not yet been introduced. If the non-milk oral water intake is less than 25ml/day, the baby is assumed to be exclusively breast-fed. We found that only two babies (PC5 and PC7) were being exclusively breast-fed; food or other supplements were 17 ml/day and 19 ml/day respectively. Thus in addition to measuring milk intake, the deuterium dilution method can be used to evaluate infant feeding practices.

CONCLUSION

The dose-to-mother deuterium oxide method has been successfully used to measure breast milk intake of Ghanaian infants. Findings also indicated that the majority of the babies were not exclusively breast-fed as claimed by the mothers.

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