Original Article

Association of 6 months of exclusive breastfeeding with higher fat-free mass in infants in a low-resource setting with high HIV prevalence in South Africa

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Exclusive breastfeeding for 6 months is recommended by the World Health Organisation (WHO) for optimal health and growth of infants, but it is not a common practice in South Africa. A breastfeeding counselling programme was run to inform, encourage and support mothers to exclusively breastfeed their infants for 6 months, and mother–infant pairs were invited to participate in a research project to determine breast milk intake volumes using the dose-to-mother deuterium dilution stable isotope technique. This technique yields objective measurements of breast milk intake volumes and also enables determination of exclusivity of breastfeeding, which is most frequently determined by maternal recall and can be subject to bias. Exclusivity of breastfeeding at 6 weeks, 3 months and 6 months following birth of the infants was correlated with infant fat-free mass at 12 months, which was determined by the dose-to-infant deuterium dilution stable isotope technique. Results showed that infants who were exclusively breastfed for 6 months had a higher per cent fat-free mass at 12 months compared with infants who were not exclusively breastfed for 6 months ($P < 0.05$). This objective determination of both breastfeeding patterns and infant body composition gives weight to the WHO recommendation of exclusive breastfeeding for 6 months as it demonstrated adequate fat-free mass in infants at 12 months, even in an area with high HIV prevalence. © 2016 John Wiley & Sons Ltd

Keywords: exclusive breastfeeding, fat-free mass, stable isotope, deuterium dilution technique, dose to infant.

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Introduction

The World Health Organisation (WHO, 2002) recommends exclusive breastfeeding for the first 6 months of an infant's life for optimal infant health and growth, and numerous studies have shown that it is beneficial for both mother and infant (Hoddinott *et al.* 2008). However, in South Africa, exclusive breastfeeding is not a common practice (Tylleskar *et al.* 2011), and despite health workers advocating exclusive breastfeeding, mothers frequently introduce complementary foods as early as 1 month (Mamabolo *et al.* 2004; Nor *et al.* 2012). The most recent Demographic and Health Survey in South Africa stated that only 8% of infants were exclusively breastfed (EBF) in the first 6 months of life (National Department of Health

South Africa 2004); the more recent South African National Health and Nutrition Examination Survey report gave a similar figure of 7.4% (Human Sciences Research Council 2012). Exclusive breastfeeding is of critical importance for child survival as another South African study showed that the mortality rate of HIV-exposed infants at 3 months was 6.1% in EBF infants compared with 15.1% in formula-fed infants (Coovadia *et al.* 2007). Formula feeding is risky especially where acceptable, feasible, affordable, sustainable and safe criteria are not met.

Countrywide surveys of exclusive breastfeeding involve collection of retrospective data from maternal recall, and these often overestimate the proportion of women exclusively breastfeeding. Several studies have been carried out on maternal recall of infant feeding

practices from retrospective data, which was compared with earlier reported feeding practices, and the retrospective data have been shown to be biased, especially in regard to duration of exclusive breastfeeding (Bland *et al.* 2003; Li *et al.* 2005; Greiner 2014). Exclusive breastfeeding, measured by maternal recall, is subject to bias, especially when mothers are educated as to the benefits of exclusive breastfeeding, and this social desirability bias has been reported in dietary self-reports (Hebert *et al.* 2008).

There has been much debate about how to accurately classify infant feeding (Hector 2011), but the method most frequently used to assess infant feeding is a 24-h recall as it reflects current infant feeding practice and is less subject to mothers not recalling correctly what was given. Our study included a 24-h recall and also classification of exclusive breastfeeding according to the dose-to-mother deuterium dilution (DTM) technique (Coward *et al.* 1982). The DTM technique is based on a two-compartment model and enables objective determination of both breast milk intake volumes and non-milk oral intake volumes (intake of water from sources other than breast milk), and hence, infants can be classified according to their exclusivity of breastfeeding. Although other methods, e.g. test weighing and dose-to-infant deuterium dilution techniques, can be used to quantify breast milk intake volumes, they are not able to classify infants according to exclusivity of breastfeeding.

Infant body composition

Studies in developed countries have looked at subsequent obesity and have focused on the differences between breastfed and formula-fed infants (Dewey

et al. 1993; Butte *et al.* 2000). A systematic review of the growth characteristics of breastfed compared with formula-fed infants showed that infants who are breastfed have a different pattern of growth – they gained less weight than formula-fed infants and generally have a lower weight at 12 months (Dewey 1998).

Measurements of body composition in infants have typically used weight and body mass index (BMI) as indicators, and this could lead to inadequate interpretation of scientific evidence. For example, a study that included measurement of fat mass and BMI at age 4 years showed that there was a significant increase in fat mass for children who had never been breastfed compared with infants who had been breastfed for 12 months or more, but there was no such association between breastfeeding and BMI (Robinson *et al.* 2009).

In a review of the methods used to determine body composition, it was reported that most techniques require the use of predictions in the determinations, and it is unsure whether these predictions are valid for all populations (Wells & Fewtrell 2006). BMI takes into account body weight and does not distinguish between fat mass and fat-free mass. Studies looking at adiposity rather than weight are thought to be more relevant in determining obesity. Skinfold thickness is another commonly used measure of infant body composition; however, skinfold thickness determines regional fatness and cannot be used in determining fat-free and fat mass in the body as a whole (Wells & Fewtrell 2006).

Deuterium dilution methods enable assessment of fat and fat-free mass and can be used for all age groups and are relatively easy to carry out (Wells & Fewtrell 2006). Our longitudinal study enabled us to look at different objectively determined early breastfeeding practices (at three time points up to 6 months) and their

Key messages

- Determination of exclusive breastfeeding from the dose-to-mother deuterium dilution technique enabled assessment of infant body composition at 12 months based on objectively measured early breastfeeding practices, rather than maternal recall.
- Mean infant per cent fat-free mass, fat-free mass and fat-free mass index at 12 months were compared with early infant breastfeeding practices, and only per cent fat-free mass was statistically significant at 6 months.
- Exclusive breastfeeding for 6 months resulted in significantly higher percentage fat-free mass in infants at 12 months compared with infants who were not exclusively breastfed for 6 months.

subsequent effect on infant body composition at 12 months using the dose-to-infant deuterium dilution stable isotope (DTI) technique, which allows determination of whole-body fat mass and fat-free mass, thereby circumventing the limitations of other methods as discussed. This is important, especially in the light of evidence that suggests that breastfed infants have a different growth pattern to non-breastfed infants (WHO 2002).

Materials and methods

Study location

The study took place at the Umkhumbane Community Health Centre, in Cato Manor, Durban (also known as Cato Manor Clinic). This peri-urban community is a low-resource setting and is characterised by high unemployment, areas with poor sanitation and a high percentage of adults who are HIV infected. The infants in this area are therefore particularly at risk from mixed feeding and formula feeding, as they often lack the necessary hygiene and safe water requirements, and therefore, breastfeeding, and in particular exclusive breastfeeding, is protective against some of the external realities faced by families in this community.

Subjects

Mothers were approached at antenatal and early post-natal visits at Cato Manor Clinic and invited to participate in a programme run by the Department of Paediatrics and Child Health of the University of KwaZulu-Natal, viz. the Improved Nutrition Programme, which ran bi-monthly for the first 6 months and thereafter monthly up to 12 months following the birth of the baby. This programme educated, encouraged and supported mothers to exclusively breastfeed their infants and covered topics such as common breastfeeding problems, correct positioning of the infant for breastfeeding, complementary feeding, general life skills and gardening. Mothers who were participating in the Improved Nutrition Programme were invited to take part in the Breast Milk Intake Study if they met the exclusion and inclusion criteria and were informed

about the study, including its longitudinal nature, which involved five study time points, viz. 6 weeks and 3, 6, 9 and 12 months. In total, 100 mother–infant pairs were enrolled who had completed at least one of the five study time points. Inclusion criteria for the mother were initially that the mother was healthy, had no HIV or other infectious disease, intended to breastfeed her baby for 12 months, was participating in the Improved Nutrition Programme, intended to live in the neighbourhood of the clinic for 12 months after delivery and is African. The number of women recruited with these criteria was low; hence, an amendment was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal to allow asymptomatic HIV-infected mothers to participate in the study from March 2013. Exclusion criteria for the mother were pregnancy and BMI < 18.5 kg m⁻². Inclusion criteria for the infant were full term and birthweight > 2.3 kg. Exclusion criteria for the infant were twins, any defect that interferes with feeding and chronic illness, e.g. congenital heart disease and cerebral palsy.

The sample size of 100 mother–infant pairs was a convenience sample for the primary outcome of the Breast Milk Intake Study, which studied the breast milk intake volumes at five different time points and the determinants of breast milk intake volumes, which included exclusivity of breastfeeding. The sample size enabled detection of ~0.5 standard deviation change in breast milk intake volumes, which is classified as a medium effect size (Cohen 1988), between the EBF and non-EBF groups (design with five repeated measurements having a compound symmetry covariance structure) at a significance level (*P*-value) of 0.05 or 5% and a power of 0.80 or 80%. The correlation between milk volume observations on the same subject at different follow-up time points was assumed to be relatively high at 0.50. The sample size calculation was performed using PASS 12 software (NCSS, LLC, Kaysville, Utah, USA).

Measurements

Breast milk intake and non-milk oral intake volumes

Breast milk intake and non-milk oral intake volumes were determined at each study time point according

to the standardised method of the DTM technique (IAEA 2010a). Briefly, the technique involves taking saliva samples from the mother and infant (1) before an accurately measured 30-g dose of deuterium oxide (99.8 atom% purity, Sercon Ltd UK, lot no. EB2039) is administered to the mother and (2) over a period of 14 days thereafter. The initial (day 0) and final (day 14) saliva samples were taken at the clinic; the saliva samples in between on days 1, 2, 3, 4 and 13 were taken by trained research assistants either at the clinic or at the mother's home, depending on the mother's preference. The deuterium enrichment in the post-dose saliva samples was then quantified compared with the pre-dose saliva samples using a Fourier transform infrared (FTIR) spectrometer. The deuterium enrichments in the mother–infant pairs over the 14-day period were then compared with model curves using the Solver function of Excel®, which minimises the sum of squares of the differences of the FTIR and model enrichments, and values for the breast milk intake and non-milk oral intake volumes for the infant are calculated from these data. The non-milk oral intake volume was then used to classify whether an infant was EBF. The model results in a small apparent intake of water from sources other than breast milk in infants who are EBF (IAEA 2010a); hence, the current cut-off for exclusive breastfeeding using this technique is 25 g day^{-1} non-milk oral intake. Only infants with non-milk oral intake of $\leq 25\text{ g day}^{-1}$ were classified as EBF; infants with non-milk oral intake $> 25\text{ g day}^{-1}$ were classified as non-EBF (IAEA 2014). Fig. 1 shows examples of the deuterium enrichment curves of infants who were thus classified as EBF (A) and non-EBF (B) using the DTM technique.

Mother's report of infant feeding (24-h recall)

Mothers used the validated WHO (1991) 24-h recall to report infant feeding, which lists possible foods that could have been given to the infant: vitamins and mineral supplements; medicine; plain water; sweetened or flavoured water; fruit juice; tea or infusion; infant formula; tinned, powdered or fresh milk; solid or semi-solid food (to be specified); oral rehydration solution;

and other (to be specified). For the purposes of the mother's reported infant feeding, classification of exclusive breastfeeding was carried out according to the WHO (2008) definition of exclusive breastfeeding, which is breast milk and vitamins and mineral supplements and medicine only (EBF). If any other category was listed, the infant was reported as not exclusively breastfeeding (non-EBF).

Anthropometric measurements and infant body composition

At each time point, infant weight and height were measured in duplicate using trained research assistants and according to the guidelines for anthropometric measurements (WHO 1995).

Infant body composition was measured using the DTI technique (IAEA 2010b), which involves giving an accurately pre-weighed dose of approximately 6 g deuterium oxide (99.8 atom% purity, Sercon Ltd UK, lot no. EB2039) to the infant to drink. The dose was weighed using a three-decimal place top pan balance (BEL Engineering, Monza, Italy) in a labelled glass bottle with a screw lid, and the exact weights were recorded. If the infant was still breastfeeding, the dose was given after the saliva sample had been taken from the infant on the last day of the breast milk intake measurement. This then served as the background or baseline saliva sample for later analysis by FTIR. If the infant was no longer breastfeeding, the infant was invited for the scheduled 12-month visit, and a saliva sample was taken before the dose was given to the infant. The infant sipped the deuterium dose directly from the bottle containing the dose, and care was taken to avoid any spillage. A pre-weighed tissue was held under the infant's mouth, and in case of spillage, the tissue was re-weighed to determine the amount of deuterium spilt. After drinking the dose, the lid was replaced on the bottle, and this was then taken to the laboratory for re-weighing. This weight was then subtracted from the weight of the bottle plus dose to determine the exact amount of deuterium that was consumed by the infant. After 3 h, a further saliva sample was taken from the infant, and this was later analysed in comparison with the pre-dose saliva sample by FTIR. Fat mass and fat-free mass were

A

Participant Study ID	4				
MOTHER'S BODY COMPOSITION					
D space (V _D), kg	28.3	% body wt	normal values	Em(0) =	1043.00 mg·kg ⁻¹
TBW, kg	27.2	47.4	40-65%	k(mm) =	0.11 day ⁻¹
Fat-Free Mass, kg	37.1	64.7	50-85%	F(bb) =	0.83 day ⁻¹
Body fat, kg	20.3	35.3	10-50%	F(bm) =	0.72 kg·day ⁻¹
Fat, %	35.3			k(bm) =	0.03 day ⁻¹
KINETIC DATA					

Human milk intake (M)	0.825	kg.day ⁻¹	825	g.day ⁻¹
Water input from milk (Fm)	0.783	kg.day ⁻¹		
Water used in growth (Fg)	0.007	kg.day ⁻¹		
Total water output (Fob)	0.835	kg.day ⁻¹		
Non-oral water intake (Fa)	0.053	kg.day ⁻¹		
Non-milk oral intake (Fs)	-0.003	kg.day ⁻¹	-3	g.day ⁻¹
Square root MSE (mg/kg)	24.54	Total error

B

Participant Study ID	25				
MOTHER'S BODY COMPOSITION					
D space (V _D), kg	36.0	% body wt	normal values	Em(0) =	839.73 mg.kg ⁻¹
TBW, kg	34.6	55.6	40-65%	k(mm) =	0.10 day ⁻¹
Fat-Free Mass, kg	47.2	75.9	50-85%	F(bb) =	0.93 day ⁻¹
Body fat, kg	15.0	24.1	10-50%	F(bm) =	0.54 kg.day ⁻¹
Fat, %	24.1			k(bm) =	0.02 day ⁻¹
Water intake, kg.d ⁻¹	3.50				
KINETIC DATA					

Human milk intake (M)	0.624	kg.day ⁻¹	624	g.day ⁻¹
Water input from milk (Fm)	0.600	kg.day ⁻¹		
Water used in growth (Fg)	-0.003	kg.day ⁻¹		
Total water output (Fob)	0.941	kg.day ⁻¹		
Non-oral water intake (Fa)	0.059	kg.day ⁻¹		
Non-milk oral intake (Fs)	0.279	kg.day ⁻¹	279	g.day ⁻¹
Square root MSE (mg/kg)	17.46	Total error

Fig. 1. Dose-to-mother deuterium dilution technique results for breast milk intake in an exclusively breastfed infant (non-milk oral intake $\leq 25 \text{ g day}^{-1}$) (A) and a non-exclusively breastfed infant (non-milk oral intake $> 25 \text{ g day}^{-1}$) (B).

calculated for the infant according to the following: the exact deuterium dose given to the infant, the deuterium enrichment in the saliva sample, the infant's weight and the appropriate hydration factor for fat-free mass, which is 0.790 for boys and 0.788 for girls at infant age of 12 months (IAEA 2010b). Fat-free mass index was calculated as infant fat-free mass (kg)/infant height (m^2). We chose to use percentage fat-free mass in this article as an indicator of a healthy infant as meta-analyses have surmised that early breastfeeding practices are protective against obesity in later life (WHO 2007). However, the percentage fat mass is simply the reciprocal of percentage fat-free mass.

Ethical considerations

Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE 211/11), and all participants were informed about the study and signed an informed consent. Stable isotopes such as deuterium have been used extensively for over half a century in metabolic studies. Deuterium is a stable isotope of hydrogen and does not emit potentially harmful radiation. The deuterium consumed by mothers in the DTM technique used in this study of breast milk intake volumes and body composition enriches body water to a maximum of 0.1% in the mother and less than half of this in her infant. No

adverse side effects have been reported at these low levels (Jones & Leatherdale 1991), and therefore, this method is not considered to be harmful to participating mothers and infants.

Statistical methods

Data were entered onto an Excel® spreadsheet and analysed using the DATA ANALYSIS TOOLPAK and OPENEPI software version 3.03. A two-sample independent *t*-test was used to compare the means of two groups with a confidence level of 95%. Kappa analysis was used to determine the level of agreement between measured (DTM technique) and reported exclusive breastfeeding using STATA version 13 (StataCorp, Texas, USA).

Results

Although a total of 100 mother–infant pairs were recruited in the study, they were not necessarily available for all time points, and therefore, the total number at each time point was less than 100. In total, after data cleaning, breast milk intake and non-milk oral intake volumes were available for 46 mother–infant pairs at 6 weeks, 74 at 3 months and 72 at 6 months. Fifty-three per cent of mothers could not be recruited at the 6-week time point, hence the smaller number of infants at the 6-week time point. For the first group of enrolled mothers, this was due to a long delay in ethical approval of the study, which meant that these mothers could not start the study at the 6-week time point.

Data cleaning focused on checking for missing entries and valid ranges. Scatter plots and histograms were constructed to highlight any high or low values and check for outliers. For the breast milk intake volumes, this resulted in one, three and two measurements being excluded at the 6-week, 3-month and 6-month time points, respectively, as the breast milk intake volumes were invalid. Two breast milk intake measurements at both 3 and 6 months found no deuterium in the infant's saliva over the 14-day period (but it was found in the mother's saliva), and these samples were therefore also excluded from subsequent analysis of breast milk intake volumes as the infant was not being

breastfed by the mother. This was also reported in Bangladesh in three mothers where little or no deuterium was detected in the infant (Moore *et al.* 2007). The number of samples excluded because of a poor fit to the model was four and one at the time points 3 and 6 months, respectively, and these levels are not unusual in a technique with such complex methodology. Some of these were due to incomplete sample collection or errors in sample collection.

Mothers were invited to return for the 12-month time point for infant body composition measurement even if they had stopped breastfeeding, which accounted for 21 infants who did not do the breast milk intake determination at 12 months, but the infant body composition was determined. Infant body composition at 12 months gave invalid values in 17 infants, and a further two infants did not do the infant body composition test; therefore, the total number of measurements available for analysis was 63. Measurements of fat mass < 7% were used to identify invalid values in line with the minimum value compatible with life (IAEA 2012). This figure is based on a review article of a study of starvation in adult men (Dulloo & Jacquet 1999), as it is unethical to conduct starvation experiments in children, and studies of weight loss in athletes (Loenneke *et al.* 2011). The cause of these low values could be the following: incomplete consumption of the dose or the assumptions made in the DTI technique, e.g. equilibration was not achieved, and invalid hydration values of fat-free mass (IAEA 2010b). In total, valid measurements for breast milk intake volume data at 6 weeks and valid infant body composition data at 12 months were available for 30 infants; valid measurements for breast milk intake volume data at 3 months and valid infant body composition data at 12 months were available for 48 infants; and valid measurements for breast milk intake volume data at 6 months and valid infant body composition data at 12 months were available for 50 infants.

Mother's reports of exclusive breastfeeding were compared with the results from the DTM technique; four reports were missing, one at 3 months and three at 6 months. Kappa analysis of measured (DTM technique) vs. reported exclusive breastfeeding showed little or no agreement at time points up to 6 months (Table 1), and over-reporting of exclusive

Table 1. Comparison of mother's report of exclusive breastfeeding with exclusive breastfeeding as determined by the dose-to-mother deuterium dilution technique

Time point	Measured agreement (%)	Expected agreement (%)	Kappa value	P-value
6 weeks	52.17	45.18	0.13	0.078
3 months	36.11	37.04	−0.01	0.605
6 months	49.30	42.11	0.12	0.031

breastfeeding was common. Table 2 shows some relevant maternal and infant characteristics of the mother–infant pairs who had valid measurements for the early infant breastfeeding practices from the DTM technique and valid measurements for the infant body composition at 12 months. The subsequent statistical analyses presented in this paper refer to these infants.

The mean percentage infant fat-free mass at 12 months was compared with the breastfeeding category as determined by the DTM technique (exclusive breastfeeding or non-exclusive breastfeeding), and the results were statistically significant at 6 months, but not at earlier time points (Table 3). The mean percentage infant fat-free mass was 6.0% higher (95% confidence limits 79.1, 87.5% EBF; 75.2, 79.4% non-EBF). Linear regression of potential confounding factors (infant gender, birthweight and maternal HIV status) on the infant's body composition at 12 months showed that the influence of these factors was all not significant.

The comparison of means for infant fat-free mass (in kg) and infant fat-free mass index (in kg m^{-2}) in relation to exclusivity of breastfeeding showed no

statistical significance at any of the time points up to 6 months (Table 3).

Discussion

Our study showed that comparisons of mean fat-free mass, fat-free mass index and per cent infant fat-free mass at 12 months according to exclusivity of breastfeeding showed a statistical difference only for mean per cent infant fat-free mass at 12 months for infants who were exclusively breastfeeding at 6 months. The EBF infants had a 6% higher mean per cent fat-free mass at 12 months, which already shows the outcome that infant body composition in later childhood could be dependent on early infant feeding practices.

Other studies have focused on fat-free mass and not per cent fat-free mass and therefore found no correlation with infant feeding mode, even where EBF infants were compared with exclusively formula-fed infants (Butte *et al.* 2000). Hence, this study has shown the value of measuring infant per cent fat-free mass for assessing the impact of objectively determined early breastfeeding practices on subsequent infant body composition.

The DTM technique provides a way to objectively assess the effects of early infant feeding on infant body composition, which is important as there was little or no correlation between mother's report of exclusive breastfeeding and exclusive breastfeeding as determined from the DTM technique. This highlights the need for objective measurement when assessing the impact of exclusive

Table 2. Maternal and infant characteristics for the infants with body composition measurements at 12 months

Time point and feeding category by DTM technique	Infant gender (male/female)	Infant birthweight (kg) ¹	Maternal HIV infected/uninfected
6 weeks EBF (<i>n</i> = 11)	7/4	3.05 ± 0.40	6/5
6 weeks non-EBF (<i>n</i> = 19)	7/12	3.23 ± 0.45	9/10
3 months EBF (<i>n</i> = 17)	8/9	3.20 ± 0.40	2/15
3 months non-EBF (<i>n</i> = 31)	17/14	3.19 ± 0.43	16/15
6 months EBF (<i>n</i> = 6)	2/4	3.09 ± 0.35	1/5
6 months non-EBF (<i>n</i> = 44)	24/20	3.20 ± 0.44	26/28

DTM, dose-to-mother deuterium dilution technique; EBF, exclusively breastfed.

¹Mean ± standard deviation.

Table 3. Infant fat-free mass, per cent fat-free mass and fat-free mass index at 12 months according to exclusivity of breastfeeding up to 6 months as determined by DTM technique

Time point and feeding category by DTM technique	Infant fat-free mass at 12 months ¹ (kg)	Mean difference (kg)	Infant fat-free mass at 12 months ¹ (%)	Mean difference (%)	Infant fat-free mass index at 12 months ¹ (kg m ⁻²)	Mean difference (kg m ⁻²)
6 weeks EBF	7.5 ± 0.8 (<i>n</i> = 11)	0.1	76.4 ± 5.4 (<i>n</i> = 11)	2.1	14.7 ± 1.1 (<i>n</i> = 10)	0.1
6 weeks non-EBF	7.6 ± 1.1 (<i>n</i> = 19)		78.5 ± 6.8 (<i>n</i> = 19)		14.6 ± 1.5 (<i>n</i> = 19)	
3 months EBF	7.6 ± 0.7 (<i>n</i> = 17)	0.1	79.3 ± 5.5 (<i>n</i> = 17)	1.2	14.5 ± 1.5 (<i>n</i> = 17)	0.0
3 months non-EBF	7.5 ± 1.1 (<i>n</i> = 31)		78.1 ± 6.8 (<i>n</i> = 31)		14.5 ± 1.8 (<i>n</i> = 27)	
6 months EBF	7.2 ± 0.7 (<i>n</i> = 6)	0.3	83.3 ± 4.0 (<i>n</i> = 6)	6.0 ²	14.2 ± 1.0 (<i>n</i> = 6)	0.3
6 months non-EBF	7.5 ± 0.9 (<i>n</i> = 44)		77.3 ± 7.0 (<i>n</i> = 44)		14.5 ± 1.7 (<i>n</i> = 41)	

DTM, dose-to-mother deuterium dilution technique; EBF, exclusively breastfed.

¹Mean ± standard deviation.²*P* < 0.05.

breastfeeding on maternal and infant outcomes. A recent article using this objective measurement of exclusivity of breastfeeding was able to show that gut inflammation was reduced with increased exclusivity of breastfeeding (Moodley-Govender *et al.* 2015). These objective measures can be used to strengthen existing programmes designed to support mothers and give scientific evidence for increased advocacy and promotion of exclusive breastfeeding for a full 6 months. However, for determining trends on exclusive breastfeeding rates in large surveys, the DTM technique would not be feasible, and thus, the rigorous application of the 24-h maternal recall is adequate.

Limitations

One limitation of the technique is that determination of breast milk intake and non-milk oral intake volumes and thus exclusive breastfeeding only represents breastfeeding practices during the 14-day period and may not be representative of the infant's breastfeeding practices outside the period of observation. Breastfeeding patterns can change according to the infant's needs and may not be constant over time (Bland *et al.* 2003), and a mother who is given infant feeding support can also change from mixed feeding to exclusive breastfeeding.

The DTM technique for determination of breast milk intake volumes uses 25-g day⁻¹ non-milk oral intake as the upper cut-off to categorise infants as

EBF. However, there are concerns that this value may be too low and this would result in an underestimation of the number of infants who are in fact being EBF.

Although care was taken during dosing of the infants, spillage in dosing of infants is a common problem with determination of infant body composition using the DTI technique and may have been responsible for some of the invalid values. Furthermore, the technique allows the infant to have a small snack 1 h after the dose, which is taken into consideration when calculating the fat-free mass, but in the case of breastfeeding infants, it is not possible to quantify the amount of breast milk that the infant consumed if the mother breastfed the infant during the 3-h period of the measurement.

A further limitation is the loss to follow-up and the small number of women who were still exclusively breastfeeding at 6 months, which resulted in a low number of objectively measured EBF infants at 6 months. This resulted in a cross-sectional analysis of the breast milk intake data at the three different time points. However, the result that exclusive breastfeeding up to 6 months resulted in a higher infant fat-free mass at 12 months remained significant even after taking other infant and maternal factors into account (infant gender, birthweight and maternal HIV status).

Conclusions

In light of increasing obesity in infants, which can persist into adulthood, our results are important as they

indicate how continued exclusive breastfeeding up to 6 months is important for infant health. Our study showed that the infants who were EBF at 6 months had a significantly higher mean per cent fat-free mass at 12 months than those who were not EBF. The DTM technique affords the opportunity for objective assessment of exclusive breastfeeding and its relation to infant body composition, as it was established that there was little or no correlation between objective assessment and maternal recall of exclusive breastfeeding.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

HM contributed to the study design, planned and conducted the study, analysed the samples and data, interpreted the results as part of a PhD undertaken at the University of KwaZulu-Natal and prepared the draft of the manuscript. AC was involved in developing the original study design, supervised the PhD research and reviewed and edited the manuscript draft.

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