

# Application of the deuterium-oxide dose-to-mother technique to determine the exclusivity of breastfeeding in women in Kanye, Botswana

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**Keywords:** breastfeeding, deuterium-oxide dose-to-mother technique, nutritional status

## Abstract

**Objective:** The objective of the study was to determine breastfeeding exclusivity and impact on the nutritional status of mothers and infants.

**Design:** This was an observational descriptive study.

**Setting:** The setting was government clinics in Kanye, Botswana.

**Subjects:** Women who gave birth to singleton healthy babies participated in the study.

**Method:** Milk intake and breastfeeding exclusivity were determined by the deuterium-oxide dose-to-mother technique. Nutritional status was assessed using anthropometric measures (height and weight) in 56 mother-infant pairs at six weeks, and again at three and six months postpartum.

**Results:** Mothers overestimated exclusive breastfeeding rates at all of the time points. However, according to the deuterium-oxide dose-to-mother technique, at three months 61% of the mothers were exclusively breastfeeding, 13% were predominantly breastfeeding, and 27% were partially breastfeeding. At six months, none of the mothers were exclusively breastfeeding. The mean body mass index (BMI) and the percentage of body fat of the mothers in all of the groups were within the healthy range of 23.9 kg/m<sup>2</sup> and 28.3%, respectively, at six months. Mean infant weight was 6.1 ± 0.95 kg, 7.6 ± 1.28 kg and 8.4 ± 1.22 kg at three, six and nine months, respectively. Infant undernutrition based on weight for age was < - 2 standard deviation. Malnutrition was 5.2% and 1.7% at six weeks and six months, respectively.

**Conclusion:** Contrary to reported high rates of breastfeeding in Botswana, low rates of exclusive breastfeeding were observed with the deuterium oxide dose-to-mother technique. Therefore, this technique can be applied as a reliable method of validating mothers' reports and their recall of exclusive breastfeeding practices. In future, the application of this method would be helpful to identify challenges in infant feeding practices, policies and programmes, all of which need further prioritisation within the Botswana context.

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S Afr J Clin Nutr 2015;28(3):128-133

## Introduction

Breastfeeding a baby exclusively for the first six months, and the continuation thereof, in addition to the introduction of appropriate solid food until 12 months and beyond, has health benefits for both the mother and child, and is an important intervention in reducing infant mortality.<sup>1</sup> Prolonged and exclusive breastfeeding has also been found to improve cognitive function, measured by intelligence quotient and teachers' academic ratings in children.<sup>2</sup> However, a recent meta-analysis linked improved child cognitive development to the mother's own cognitive and socio-economic factors and situation effects.<sup>3</sup> Exclusive breastfeeding has also been associated with a reduced risk of sudden infant death syndrome.<sup>4</sup> Although breastfed infants have been reported to be less susceptible to obesity,<sup>5</sup> a recent systematic review of the literature on the effects of breastfeeding

on childhood overweight and obesity was inconclusive.<sup>6</sup> Finally, and most importantly, breast milk is available at the right temperature, is a sterile product and is free. Mothers who breastfeed are also less likely to suffer from breast and ovarian cancer,<sup>7,8</sup> and are 50% less likely to experience postnatal depression.<sup>9</sup> Furthermore, breastfeeding has been found to be conducive to shrinkage of the uterus postnatally, and also to assisting maternal weight loss.<sup>10</sup>

There is a dearth of data on breastfeeding practices in Botswana. It was reported in the Multiple Indicator Cluster Survey, conducted in 2000, that 29% of women exclusively breastfed for up to four months.<sup>11</sup> The high rate of human immunodeficiency virus (HIV) infection in the country could be contributing to low exclusive breastfeeding rates, because since 2001, the government of Botswana has provided infant formula free of charge to HIV-infected

mothers. The data suggest that there has been a considerable decline in exclusive breastfeeding rates with the advent of free formula provision for HIV-infected women.<sup>12</sup> Therefore, it is important to invest in efforts to promote and support exclusive breastfeeding. The process for priority setting must start with an assessment of the exclusivity of breastfeeding, an analysis of the determinants thereof and the challenges faced by lactating women in their environment.

Maternal reports of breastfeeding, and especially cross-sectional reports, are known to be inaccurate in terms of actual practice.<sup>13</sup> Therefore, it is important that the application of more accurate methods with which to determine breastfeeding exclusivity is considered. The deuterium-oxide dose-to-mother technique is a method which has recently been recommended.<sup>14</sup> This method offers an opportunity to determine maternal body composition and breast milk output concurrently.<sup>15</sup> In addition, the deuterium-oxide dose-to-mother technique is a noninvasive, simple, safe, accurate and reproducible method used to measure breast milk intake, especially in non-exclusively breastfed infants.<sup>16</sup> The present study aimed to determine the exclusivity of breastfeeding and its impact on the nutritional status of mothers and infants using the deuterium-oxide dose-to-mother technique. This study was part of the International Atomic Energy Agency (IAEA) project (RAF 6039) entitled *Applying stable isotopes to monitor and improve infant and young child interventions in the Africa region*, and dubbed the African Cooperative Agreement for Research, Development and Training.

## Method

### Study design and participants

This study applied observational descriptive analyses conducted on 56 mother and baby pairs. Women were recruited at 28 weeks of their pregnancy when they reported for their antenatal visit at any of the six government clinics in Kanye. After an explanation of the study was provided, the study participants had the option of whether or not to participate. This was documented through signed consent forms. Eligibility criteria included HIV-negative women aged 18-35 years who were willing to breastfeed for at least six months, as well as attend scheduled baby clinic visits and permit home visits, with a singleton pregnancy and a gestational age of  $\geq 36$  weeks. Women who delivered by Caesarian section, or with conditions requiring specialised medical care, i.e. those with cardiac or chronic respiratory problems, were excluded from participating. The promotion and support of exclusive breastfeeding according to Botswana government protocol was performed by trained breastfeeding counsellors during home and clinic visits. Mothers were given educational booklets especially designed for the study on infant feeding, printed in both English and Setswana as support materials after the counselling sessions. Visits were made two days post delivery at their homes, and then at the clinics at six weeks, and then at three and six months. Ethical approval (HRDC 00542) was obtained from the Ethics Review Committee of the Ministry of Health in Botswana prior to study commencement. Quality control measures included the use of a standardised questionnaire, training interviewers to ensure high accuracy and precision when taking the anthropometric measurements of the study participants throughout

the study period, and when checking the questionnaires after every scheduled visit by the supervisor. All study protocol was pre-tested before commencement of the study.

### Socio-demographic questionnaire

Socio-demographic information, including data on education, employment, type of dwelling, household amenities and marital status, was collected by means of a questionnaire.

### Anthropometric measurements

The infant's weight was measured using a digital paediatric scale (Seca® 376), while wearing minimal clothes (undergarments only) to the nearest  $\pm 0.01$  kg, and expressed as a weight-for-age z-score relative to the World Health Organization (WHO) Child Growth Standards.<sup>17</sup> Infant length was measured using a Holtain® infantometer. All measurements were taken in duplicate, and the average used in the analysis. The infants were classified as underweight, wasted or stunted if their weight for age, weight for height and height for age were  $< -2$  standard deviation (SD) of the WHO Child Growth Standards. While wearing minimal clothes, the mothers were weighed to the nearest 0.01 kg using an electronic scale (Seca® 876). The height of the mothers, without shoes, was measured to the nearest 0.1 cm, using a Detecto stadiometer. Body mass index (BMI) was then calculated as weight (kg)/height (m<sup>2</sup>). Mothers were classified as undernourished if their BMI was  $< 18.5$  kg/m<sup>2</sup>.

### Infant feeding practice questionnaire

Non-breast milk liquid and complementary food intake was assessed using the 24-hour recall questionnaire. Mothers were asked to recall the number of days on which other liquid or food was given. The mothers were asked whether or not their infant had received any of the listed liquids and foods since birth. In addition, the mother was asked to estimate the volume (using a 250 ml cup) of non-breast milk liquid and number of spoons of solid food eaten during a meal. The frequency of breastfeeding was also assessed in the questionnaire. The validity of the answers was determined by probing for information on infant feeding practices not otherwise reported, as well as on breastfeeding difficulties.

### Assessment of human milk intake using deuterium-oxide dose to mother technique

Breast milk intake was measured using the deuterium-oxide dose given to the mother, based on the IAEA standard procedure. Deuterium oxide is a water-labelled stable isotope of hydrogen, and does not pose a hazard to mothers nor infants.

Human milk intake was determined by the deuterium-oxide dose-to-mother technique.<sup>18</sup> A baseline saliva sample (2 ml) was collected from the mothers by instructing them to chew on cotton wool prior to dose administration. Subsequently, a dose of 30 g accurately weighed deuterium oxide was administered to the mothers from a bottle using a drinking straw to avoid spillage. The bottle was rinsed with 50 ml of tap water and given to the mother to drink to ensure complete ingestion of the dose. Further saliva samples were then collected from the mothers and the babies one, two,

three, four, 13 and 14 days after the dose was taken, in accordance with the standard methodology used when deuterium oxide is used to determine breastfeeding exclusivity and the volume of milk consumed. The saliva samples were placed in a cool box (4° C) and transported to an IAEA-designated laboratory at the National Food Technology Research Centre, Kanye, Botswana, and stored at – 20° C until analysis. Saliva sample bottles were clearly labelled, indicating the date and times that they were collected, as well as the participants' codes.

Deuterium-oxide enrichment in the saliva samples was measured using a Fourier transform infrared spectrophotometer, i.e. FTIR-8400S®. Absorbance was determined to be from 2 300-28 00 cm<sup>-1</sup>. The intake of breast milk and water from non-milk sources was calculated by fitting the isotopic data to a model for water turnover in the mothers and infants, and measuring the transfer of milk from the mother to the infant<sup>19,20</sup> using an algorithm (2007 Excel® spreadsheet) developed by the Medical Research Council Collaborative Centre for Human Nutrition Research, Cambridge, UK.

Data on non-breast milk water intake were grouped into three feeding categories in alignment with the study reported by Haisma et al,<sup>19</sup> i.e. exclusive breastfeeding (a non-breast milk intake < 52 ml/day), predominant breastfeeding (a non-breast milk water intake < 52-216 ml/day) and partial breastfeeding (a non-breast milk intake > 216 ml/day).

### Maternal body composition

The same dose of deuterium oxide given to the mother to measure the breast milk intake also served to measure total maternal body water. The mother's total body water (TBW) in kilograms was calculated using the back extrapolation technique.<sup>18</sup> Fat free mass (FFM) in kilograms was determined from the TBW using a non-aqueous exchange factor of 1.041. The fat mass in kilograms was calculated from the difference between the mother's body weight and the FFM.

### Statistical analysis

Data analysis was carried out using SPSS® version 18.0. Descriptive statistics were computed for continuous variables (mean ± SD) and categorical variables (%). The infants' z-scores (weight for age, length for age, weight for length and BMI for age) were calculated using Anthro® 2000, version 2.<sup>21</sup> The normal z-scores were considered to be between – 2 and + 2 SD of the mean. Weight and height were used to calculate adult BMI. The BMI index of the mothers and the z-scores of the infants were used to determine the nutritional status at different periods, i.e. at six weeks, and then at three months and six months.

## Results

### Socio-demographic characteristics

The socio demographic characteristics of the study sample are summarised in Table I. The age of the mothers ranged from 17-35 years, the majority had a secondary education, were of Christian faith, single and unemployed.

**Table I:** The socio-demographic characteristics of mothers who participated in the study

Age range	17-35 years
<b>Education level</b>	
Primary	2 (3.5)*
Secondary	52 (92.1)
Post secondary	1 (1.8)
Other	2 (3.5)
<b>Religion</b>	
Christian	45 (78.9)
Other	12 (21.1)
<b>Marital status</b>	
Single	50 (87.7)
Married	7 (12.3)
<b>Employment status</b>	
Unemployed	48 (84.2)
Self-employed	1 (1.8)
Wage earner	7 (12.3)
Housewife	1 (1.8)
<b>Accommodation</b>	
Rented	3 (5.3)
Owned	4 (7.0)
Other	50 (87.7)

\* Values shown as n (%)

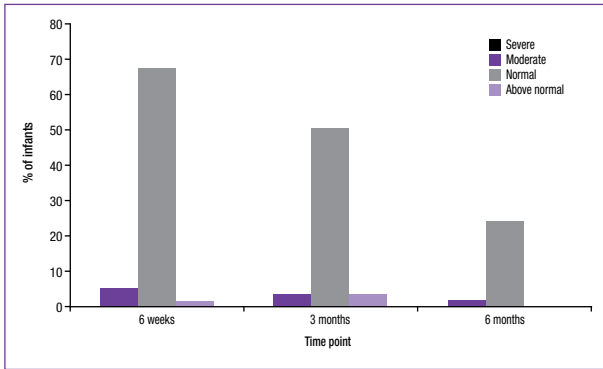
**Table II:** Maternal anthropometry and body composition

Descriptor	6 weeks (n = 56)	3 months (n = 36)	6 months (n = 28)
Height (m)	1.6 ± 5.9	1.6 ± 6.0	1.6 ± 5.6
Weight (kg)	62.1 ± 11.1	62.6 ± 12.4	62.5 ± 13.8
Body mass index (kg/m <sup>2</sup> )	24.4 ± 4.0	25.1 ± 4.8	23.9 ± 7.2
Mid-upper-arm circumference (m)	24.7 ± 5.2	25.7 ± 3.7	25.3 ± 4.6
Fat-free mass (kg)	45.5 ± 5.5	43.1 ± 8.5	42.9 ± 4.0
Percentage of body fat	24.8 ± 13.1	28.7 ± 12.5	28.3 ± 11.5

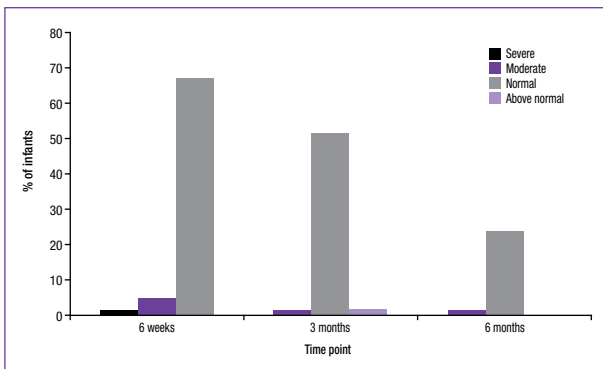
Values are mean ± SD

### Anthropometric characteristics and mothers' body composition

The mothers' anthropometric characteristics and body composition are shown in Table II. The mean BMI was 24.4 (± 4.5 kg/m<sup>2</sup>) at six weeks and 23.9 (± 7.2 kg/m<sup>2</sup>) at six months postpartum. There was a significant difference in the percentage of body fat of the mothers during the six months' follow-up (p-value 0.000). The mean FFM decreased from 45.5 kg at six weeks to 42.9 kg at six months, while the percentage of body fat increased from 24.8% at six weeks to 28.3% at six months. The infants' anthropometric data reflected moderate malnutrition, i.e. stunting, with a z-score range of –2.1 to 3.3, and underweight, with a z-score ranging from –2.8 to 5.8, at three months and six months (weight-for-age z-score of –2.1 to 1.8) and height-for-age z-score of –2.3 to 1.74), respectively.



**Figure 1:** Prevalence of underweight in the infants according to the weight-for-age z-scores



**Figure 2:** Prevalence of stunting in the infants according to the height-for-age z-scores

The prevalence of the different forms of malnutrition are shown in Figures 1 and 2.

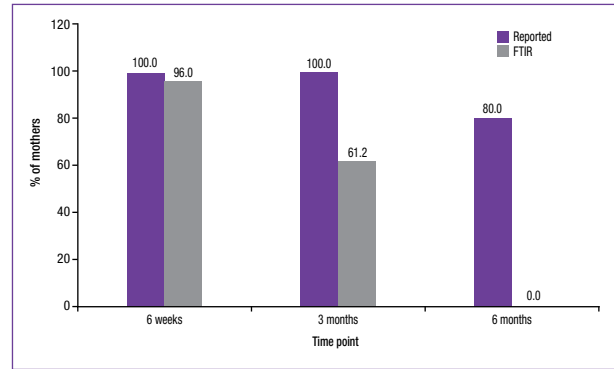
**The intake of human milk and fluids other than human milk by feeding pattern**

Table III shows the mean intake of breast milk, non-breast milk water and total water intake at each time point (at six weeks, and then at three and six months) by feeding pattern. The mean breast milk intake, measured by the deuterium-oxide dose-to-mother technique at six weeks, and at three and six months, was 611.20 + 188.09 ml/day, 791.29 + 283.44 ml/day and 838.09 + 248.09 ml/day, respectively. According to the deuterium-oxide

**Table III:** The intake of breast milk and other liquids according to the deuterium-oxide dose-to-mother technique, by feeding pattern

Age	n	Breast milk intake (ml/day (mean ± SD))	Non-breast milk intake (ml/day) (mean ± SD)	Total water intake (ml/day) (mean ± SD)
<b>6 weeks</b>	<b>50</b>	<b>611.20 ± 188.09</b>	<b>-65.82 ± 68.85</b>	<b>878.83 ± 100.92</b>
Exclusively breastfed	48	605.38 ± 187.30	-70.48 ± 64.99	876.99 ± 101.32
Predominantly breastfed	2	742.00 ± 116.67	61.00 ± 7.07	923.06 ± 111.38
<b>3 months</b>	<b>31</b>	<b>791.29 ± 283.44</b>	<b>58.32 ± 159.84</b>	<b>919.59 ± 92.36</b>
Exclusively breastfed	19	859.79 ± 288.69	-52.26 ± 66.65	902.40 ± 83.55
Predominantly breastfed	4	689.25 ± 45.39	145.50 ± 65.10	927.05 ± 111.84
Partially breastfed	8	806.13 ± 338.95	806.12 ± 338.95	956.68 ± 104.12
<b>6 months</b>	<b>23</b>	<b>838.09 ± 248.09</b>	<b>394.52 ± 215.26</b>	<b>938.91 ± 94.73</b>
Predominantly breastfed	5	701.8 ± 178.70	953.97 ± 93.66	968.97 ± 92.79
Partially breastfed	18	884 ± 244.69	875.71 ± 69.28	875.71 ± 69.28

SD: standard deviation



FIR: Fourier transform infrared spectroscopy

**Figure 3:** A comparison of breastfeeding patterns using dietary recall and those using the deuterium-oxide dose-to-mother technique

dose-to-mother technique, 48 infants at the age of six weeks were classified as exclusively breastfed (a non-breast milk oral intake of < 52 ml/day), and two as predominantly breastfed (a non-breast milk oral intake of 53-216 ml/day). At three months, 19 infants were exclusively breastfed, four were predominantly breastfed and eight partially breastfed (a non-breast milk oral intake of > 216 ml/day). At six months, none of the infants was exclusively breastfed. However, 20 were predominantly breastfed and eight partially breastfed.

Figure 3 shows the comparison between dietary recall and the deuterium-oxide dose-to-mother technique (Fourier transform infrared spectroscopy) at six weeks, and then at three and six months. Differences in the breastfeeding pattern were observed at each time point. A 4% difference was noted between the two methods in reporting exclusive breastfeeding at six weeks. Of the mothers who claimed to be exclusively breastfeeding at three months, only 61% were actually practising EBF, 13% were predominantly breastfeeding and 27% partially breastfeeding. None of the mothers were reported to be breastfeeding exclusively at six months according to the deuterium-oxide dose-to-mother technique, compared with 80% of the mothers who reported that they were exclusively breastfeeding.

**Discussion**

The present study was the first to use the deuterium-oxide dose-to-mother technique to investigate the exclusivity of breastfeeding

in women in Botswana. Previous studies in Botswana have demonstrated<sup>11,22</sup> that 29% and 23.3% of children in Botswana were exclusively breastfed at six months. Although 80% of the mothers in this study reported that they exclusively breastfed their infants at six months, according to the deuterium-oxide dose-to-mother technique, 0% were exclusively breastfeeding at this time.

Reports of the low rates of exclusive breastfeeding observed in this study are similar to observations in Morocco, where only 12.5% of the women were breastfeeding exclusively at six months,<sup>23</sup> and in South Africa, where the South African Demographic and Health Survey<sup>24</sup> of 2003 reflected that only 8.3% of infants were breastfed exclusively in the first three months. To the contrary, other African countries have reported higher rates of exclusive breastfeeding, such as Swaziland, where the rate of exclusive breastfeeding stood at 32% in infants aged six months and younger,<sup>25</sup> and similarly, Ghana, where the rate of exclusive breastfeeding was reported to be 52%.<sup>26</sup> Other countries have shown notable gains in increasing exclusive breastfeeding rates, including Malawi, where exclusive breastfeeding rose from 5% in 1990 to 50% in 2010; Sri Lanka, where it increased from 21% in 1990 to 61% in 2010; and Cape Verde, where it went from 11% in the same period.<sup>27</sup>

It is interesting to note that the rate of exclusive breastfeeding remained low in Botswana despite the mothers receiving antenatal and postnatal advice on infant and young child feeding (IYCF) practices, with particular focus on the benefits of breastfeeding during the study period. This is a clear indication that more innovative and culturally acceptable communication strategies need to be applied by policy-makers to family members and lactating mothers in order to promote exclusive breastfeeding. Breast milk is uniquely suited to human infants' nutritional needs, and has unparalleled immunological and anti-inflammatory properties which protect against a host of illnesses and diseases in mothers and their children.<sup>27</sup> However, several studies have shown that mothers find it difficult to adhere to the WHO recommendations for continued and exclusive breastfeeding, especially up to six months, even when breastfeeding initiation rates are high.<sup>28,29</sup> Although the factors that contribute to this were not documented in this study, low rates of feeding breast milk exclusivity and the duration of breastfeeding have been associated with several factors. Some of these include a fear that human milk is insufficient; ignorance, i.e. lack of knowledge of the benefits of breastfeeding on the health, performance and productivity of mothers, the necessity of working outside the home, aesthetic concerns, an absence of psychological support and a belief that breastfeeding restricts women socially.<sup>23</sup>

The deuterium-oxide dose-to-mother technique made it possible for the human milk and non-breast milk water intake to be quantified, and was used to verify the mothers' declarations. A large difference was found between the two methods, i.e. 80% versus 0% at six months, respectively. This has also been reported in previous studies.<sup>20,30</sup> The differences may be attributed to the presence of social desirability and social approval bias, which are tendencies by individuals to respond in a manner that is consistent with expected norms. Establishment of the volume of breast milk intake is of particular relevance when estimating the adequacy of nutrient intake

in exclusively breastfed infants.<sup>31</sup> Advantages of the deuterium-oxide dose-to-mother technique are the noninvasive character of the measurements, the relative ease with which it can be applied and the possibility of combining this type of evaluation with other nutritional indices.<sup>32</sup> The breast milk intake in the current study ranged from 611.2 ml/day at six weeks, 791.3 ml/day at three months and 838.1 ml/day at six months, which is within the normal range of human milk intake, according to WHO and The United Nations Children's Fund.<sup>33</sup>

The mothers in this study were within the normal cut-off range for BMI. The mean BMI and the percentage of body fat of the mothers were within the healthy range, irrespective of the breastfeeding pattern, i.e. 24.7 kg/m<sup>2</sup> and 28.7-30.7%, respectively. This is probably because Kanye (the study location) is an urban village, and the majority of the population has access to adequate resources, including water, sanitation and health care, and experiences a high standard of living. This helps Kanye residents to maintain a good nutritional status. A study in Kenya revealed that better-nourished mothers lived in an environment with better access to food and health care.<sup>15</sup> In this study, the percentage of body fat of the mothers increased from 24.8% at six weeks to 28.3% at six months. This is contrary to published data that have indicated a decrease in body fat during the first six months of lactation following childbirth.<sup>34</sup> However, changes in a mother's body composition depend on factors such as weight gain before birth, physical activity level, the stage of lactation and the mother's nutritional status, as well as food availability and cultural practices.<sup>35</sup>

Stunting and underweight were observed in the infants who participated in this study. Common nutrition problems in Botswana include protein energy malnutrition, micronutrient deficiencies and diet-related noncommunicable diseases.<sup>36</sup> Causes of nutrition-related problems include an inadequate food intake, inadequate maternal and child caring practices, and predisposing diseases, such as tuberculosis, and HIV/acquired immune deficiency syndrome.<sup>22</sup> Data from the Botswana Family Health Survey 2007 were analysed based on the 2006 WHO Child Growth Standards, and indicated that 11.9%, 31.2% and 8.6% of children were underweight, stunted and wasted, respectively.<sup>35</sup> These results are an indication that considerably more effort is needed in Botswana with respect to implementation of the Global Strategy for IYCF, adopted by The World Health Assembly in May 2002. The target of this strategy is to achieve optimal IYCF and improve IYCF practices at national level, in order to contribute to the prevention of child malnutrition and to reduce infant and young child morbidity and mortality.<sup>37</sup> Botswana has one of the most comprehensive maternal and child health services in sub-Saharan Africa. Nearly 95% of pregnant women receive prenatal care and deliver their infants with the assistance of a health professional in a health facility.<sup>38</sup> Regardless of their HIV status, during prenatal visits pregnant women are provided with education and counselling, according to the country's IYCF guidelines to ensure that they make informed and appropriate infant feeding choices.

## Conclusion

In conclusion, the results of this study demonstrate that exclusive breastfeeding in infants up to six months of age is very low in



Botswana, as determined by the deuterium-oxide dose-to-mother technique. This technique can be used to provide quantitative information on the intake of human milk by breastfed infants, as well as on the intake of water from sources other than human milk. Thus, it can be used to assess exclusive breastfeeding. Therefore, the results of this study necessitate exploration of other avenues to encourage mothers to appreciate the benefits of exclusive breastfeeding, especially in the first six months of their infant's life, as recommended by the WHO. There is also a need to continue to investigate barriers to the practice. Finally, the benefits of exclusive breastfeeding should be seen as beneficial to mothers' and infants' optimal well-being.

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