Human Breast Milk Assessed by the Deuterium Dose Mother Technique with less than 30 g Deuterium Using the Agilent 4500S for Fourier Transform Infrared Spectroscopy: A Pilot Study in the Democratic Republic of Congo

Christine Chimanuka Murhima’Alika1,3, Ghislain Maheshe Balemba1,4, Bugugu Mapendo Daniella1,3, Aminata Kapapa1,3, Grace Munthali5, Victor Owino6, Michèle Dramaix2, Philippe Donnen2 and Ghislain Bisimwa Balaluka1,3

1 Ecole Régionale de Santé Publique, Université Catholique de Bukavu, Bukavu, Democratic Republic of Congo (DRC); 2 Ecole de Santé Publique, Université Libre de Bruxelles , Brussels, Belgium; 3 Centre de Recherche en Sciences Naturelles, Lwiro, DRC; 4 Hôpital Provincial General de Reference de Bukavu, Université Catholique de Bukavu, DRC; 5 National Institute for Scientific and Industrial Research of Zambia (NISIR); 6. International Atomic Energy Agency.

Institutional and addresses for authors:

- Christine Chimanuka Murhima’Alika (MD). Ecole Régionale de Santé Publique, Université Catholique de Bukavu and Centre de Recherche en Sciences Naturelles de Lwiro (R.D.Congo). E-mail: christinechimanuka@gmail.com

- Ghislain Maheshe Balemba (MD, radiodiagnostic). Faculty of Medicine, Université Catholique de Bukavu and Hôpital Provincial General de Référence de Bukavu (R.D.Congo). E-mail: mahesheghislain@gmail.com.

- Daniella Bugugu Mapendo (DBM). Ecole Régionale de Santé Publique, Université Catholique de Bukavu and Centre de Recherche en Sciences Naturelles de Lwiro (R.D.Congo). E-mail: daniellabgg@gmail.com.

- Aminata Kapapa (MD). Ecole Régionale de Santé Publique, Université Catholique de Bukavu and Centre de Recherche en Sciences Naturelles de Lwiro (R.D.Congo). E-mail: kapapaaminata@gmail.com.
• Grace Munthali. National Institute for Scientific and Industrial Research (NISIR). Zambia. E-mail: munthaligrace@hotmail.com

• Victor Owino Ochieng (PhD, Nutritionist). International Atomic Energy Agency. Vienna, Austria. E-mail: V.owino@iaea.org

• Michèle Dramaix Wilmet (PhD, statistics). Ecole de Santé Publique. Université Libre de Bruxelles. Bruxelles, Belgique. miwilmet@ulb.ac.be

• Philippe Donnen (MD, PhD). Ecole de Santé Publique, Université Libre de Bruxelles. Belgique. E-mail: philippe.donnen@ulb.be

• Ghislain Bisimwa Balaluka (MD, PhD). Ecole Régionale de Santé Publique, Université Catholique de Bukavu and Centre de Recherche en Sciences Naturelles de Lwiro (R.D.Congo). E-mail: ghislainbiba@yahoo.fr; bisimwa.balaluka@ucbukavu.ac.cd

**Corresponding author**

Christine Chimanuka Murhima’Alika (MD). Ecole Régionale de Santé Publique, Université Catholique de Bukavu and Centre de Recherche en Sciences Naturelles de Lwiro (R.D.Congo). E-mail: christinechimanuka@gmail.com
ABSTRACT

Background:
Good breastfeeding practice is essential in preventing malnutrition in children. The deuterium method is the best technique for assessing human breast milk output. We investigated whether deuterium doses < 30 g can be used to assess human breast milk using saliva and the new Agilent 4500S for Fourier transform infrared spectroscopy (FTIR).

Methods:
In a cross-sectional study, the breastmilk output of lactating mothers was evaluated using four different doses of deuterium in the Miti-Murhesa Health Zone (Democratic Republic of Congo). Lactating mothers of infants aged 3-23 months were recruited and assigned to a dosage group. Weight, height, and mid-upper arm circumference were measured and the body mass index calculated as weight/height^2. Body composition (fat mass, free fat mass, total body water, and breastmilk output) was assessed using standard deuterium dilution methods. One-way ANOVA was used to compare the means in different groups and the chi^2 test to compare proportions.

Results:
Seventy-five lactating mothers were included in the study: 19 received 6 g, 20 received 10 g, 18 received 15 g, and 18 received 20 g deuterium. The mean ± standard deviation infant age was 10.6 ± 5.2, 11.1 ± 4.4, 10.9 ± 4.4, and 11.0 ± 3.9 months, respectively (p=0.076). We found no difference in the age and anthropometric parameters of the lactating mothers in the different groups. The mean ± standard deviation breast milk output rate was 796.6 ± 292.4, 260.8 ± 23.2, 749.8 ± 244.2, and 733.6 ± 207.1 g/d in the 6 g, 10 g, 15 g, and 20 g groups, respectively (p=0.883).
**Conclusions:**

The different doses of deuterium administered to lactating mothers resulted in similar breast milk output values. Thus, it is possible to evaluate human breast milk with deuterium doses $<30$ g when using the Agilent 4500S.

**Key words:** Breast milk output, deuterium dilution, lactating mother, body composition, D.R.Congo.
BACKGROUND

Malnutrition is a public health problem, especially in low and medium-income countries (LMICs). According to the latest Global Nutrition Report, the prevalence of stunting in children aged 6-59 months is 22.2%, accounting for 150.8 million children, whereas 50.3 million (7.5%) suffer from wasting [1,2]. In the short term, malnutrition can lead to death, and an estimated 45% of 5.9 million deaths in children under 5 years of age worldwide are attributable to malnutrition [3], though the total number of deaths worldwide fell by 52.5% between 1990 and 2015 [4].

Good breastfeeding practice is one of the essential strategies to prevent infant malnutrition and the WHO recommends the exclusive breastfeeding strategy for the first six months of life [5]. Several authors have documented the nutritional, health and socioeconomic benefits of breastfeeding [6].

Breast milk studies are needed to develop strategies to improve breastfeeding practices in a context in which childhood malnutrition remains a real public health problem [7,8,9]. Therefore, it is important to agree on more efficient measurement techniques. The mother-to-breastfed infant weighing method has low precision due to small variations in weight before and after breastfeeding [10].

The deuterium dilution method is currently recognized as one of the best methods for assessing the body composition of humans [11] and human milk intake or exclusive breastfeeding [12] in community settings. In the assessment of body composition, a 6 g dose of deuterium oxide (D2O) is given to the mother and saliva collected before and 3-4 hours after administration. For
breast milk, we need to collect a total of seven samples between days 1 and 14 after administering 30 g of deuterium to the mother.

The original method for measuring breast milk output was developed in the 1980s for use with isotope ratio mass spectrometry [13]. The use of Fourier transform infrared spectrophotometry (FTIR) for measuring body water spaces was introduced in the 1990s [14], and Shimadzu FTIR was no longer used in the analysis of breast milk intake. The dose was based on the limit of quantitation.

The dose depends on the method of analysis, the sensitivity of the device used to detect deuterium in saliva on day 14, and the intensity of the infant's breastfeeding, allowing good enrichment of the child's saliva on day 14. As isotope ratio mass spectrometry (IRMS) is much more sensitive than the current Shimadzu FTIR, a team of researchers used a dose of 10 g of D2O instead of 30 g to assess breast milk intake [15]. In the same logic, the detection of deuterium also depends on the body volume of the mother and breastfed child. Thus, strong lactating mothers with large babies require a higher dose of deuterium than lactating mothers and breastfed infants with low body weight.

Progress has been made in FTIR equipment, and the latest FTIR, Agilent 4500S, is more sensitive than the Shimadzu model commonly used until now [16]. The aim of our study was to determine whether deuterium doses < 30 g can be used to assess human breast milk using saliva and the new Agilent 4500S for FTIR.
MATERIALS AND METHODS

Region of study

The study took place in the health zone (HZ) of Miti-Murhesa in the province of South Kivu in the eastern Democratic Republic of Congo (DRC). The central office of the HZ is located 9 km from the Natural Sciences Research Center of Lwiro (CRSN) within which the Lwiro Pediatric Hospital (LPH) operates. The HZs are located between 1500 and 2000 m above sea level; Katana is on the shore of Lake Kivu and Miti-Murhesa is located along Kahuzi-Biega Park, which is recognized worldwide for the species of mountain gorilla it shelters. The center is best known for research in nutrition, and work is underway to study the long-term effects of malnutrition. Malnutrition has been endemic in the region for several decades even though malnutrition has increased throughout the DRC based on national surveys carried out over the last 20 years [17,18].

Study design

We designed a cross-sectional study to analyze breastmilk intake by lactating mothers using different doses of deuterium (6, 10, 15, and 20 g) and the deuterium dose mother technique (DDMT). Data collection was carried out from March 15 to April 30, 2020.
Identification of subjects

The subjects were identified by a team of four doctors, including the study coordinator, two nutritionists, two assistant nutritionists, and eight community health workers (CHWs). Lactating mothers whose infants were aged 4-23 months were identified in different health areas and villages and referred to the four targeted health centers (Buhandahanda, Chegera, Kavumu, Mulungu) for DDMT.

Sample size calculation

The sample size was determined on the basis of convenience, limitations on the amount of available D$_2$O, and the number of lactating mothers identified during the study period in the targeted health areas who agreed to participate in the study. Mothers were recruited and assigned to four deuterium dosage groups. We set a minimum of 15 subjects per group for convenience. Other researchers have reported values obtained with sample sizes of less than 15 [19,20].

Anthropometric measurements

For breastfed infants, body weight was measured to the nearest 100 g using the salter scale while the child was wearing panties. For lactating mothers, body weight was measured to the nearest 100 g using an electronic scale (OMRON, HN-289-EBK) while the subject was dressed only in light clothing. Height was determined without shoes to the nearest 0.1 cm using a SECA 206 cm® measuring tape attached to a wall. The mother’s mid-upper arm circumference (MUAC) was measured using an adult MUAC tape. The anthropometric measurements were carried out in accordance with WHO guidelines [21] and subjected to quality control by...
collecting independent measurements by two members of the team. The final measurement
was the average of the two. In the case of deviation of more than 300 g for the weight and 0.5
cm for the height, a third measurement was taken. The average of the two closest
measurements was used. Weight and height were used to calculate the body mass index (BMI)
as weight/height$^2$.

Breast milk output and mother’s body composition

The mothers’ body composition and breast milk output were determined by the DDMT.
Different doses of D$_2$O (99.8 atom % D, Cambridge Isotope Laboratories Inc., Andover, USA)
were accurately weighed to the nearest 0.001 g in sterile leak-proof autoclavable
polypropylene dose bottles on a calibrated analytical balance (Sartorius 0.0001 g; Sartorius AG,
Goettingen, Germany) at the Laboratory of the Hôpital Provincial Général de Référence Bukavu
(University Clinic of Catholic University of Bukavu, supported by the IAEA Project RAF6052 and
CRSN Lwiro). After consenting, baseline saliva samples were collected from the mother and the
baby. The mother was asked to rotate a small ball of cotton wool around their mouth until it
was completely soaked with saliva. The soaked cotton ball was then squeezed through a 10-mL
sterile disposable syringe into a 3.6-mL sterile cryovial labeled with the mother’s code, the date,
and the time of sample collection. Thereafter, saliva samples were collected from the babies
by a trained technician using a cotton wool swab wrapped with extra cotton wool. The saliva
was collected by moving the swab around the baby’s mouth until it was completely soaked with
saliva.

Post-dose saliva samples were collected from the mothers and infants as described above on
days 1, 2, 3, 4, 13, and 14 after the dose was consumed. Deuterium abundance in the saliva
was measured using an Agilent 4500S FTIR spectrometer (Agilent Technologies, 2018, Malaysia) and the enrichment calculated by subtracting the value of the baseline sample from the value of the post-dose sample. The calculated D₂O enrichment was used to calculate maternal body composition and breastmilk output.

Breast milk output was determined based on a two-compartment model of kinetics as described by Coward et al. [13,14] by fitting the isotopic enrichment data to a model for water turnover in the mothers and infants [22,23]. Maternal body composition was determined from the mother’s total body water (TBW), calculated from the y-intercept of the mother’s isotope elimination curve and the weight of D₂O consumed, and corrected for non-aqueous isotope exchange [12]. The fat free mass (FFM) was determined by dividing TBW by 0.732, the assumed hydration of the FFM. Fat mass was the difference between body weight and the FFM. Curve fitting and calculation of the output was achieved using a spreadsheet template provided by the IAEA.

Data analysis

The data were encoded in Microsoft Excel. SPSS 23.0 software was used to analyze the data. The quantitative data were presented as mean and standard deviation (SD) and categorical data as numbers and percentages. Statistical tests were applied to compare the anthropometric values of mothers and children, as well as body composition and breastmilk output in different groups. One-way ANOVA was used to compare the means in different groups and the chi² test to compare proportions.
RESULTS

A total of 75 lactating mothers with infants aged 3 to 23 months were included in the study (Table 1). However, one mother-child pair was excluded because the child’s saliva did not comply. We suspected that the mother did not drink the deuterium dose because there was no trace of deuterium in either the mother’s or the infant’s saliva. We found no difference in the age and anthropometric parameters of the lactating mothers in the different groups. The mother’s body composition and breast milk output are described in Table 2. We observed no significant difference between the different groups regarding breast milk output.

Table 1. Characteristics of the mothers and infants

| Characteristic | DDMT-6g (n=19) | DDMT-10g (n=20) | DDMT-15g (n=18) | DDMT-20g (n=18) | P-value |
|----------------|----------------|-----------------|-----------------|-----------------|---------|
| Infants        |                |                 |                 |                 |         |
| Age, months    | 10.6 ± 5.2     | 11.1 ± 4.4      | 10.9 ± 4.4      | 11.0 ± 3.9      | 0.980   |
| Female, %      | 57.9           | 60.0            | 66.7            | 50.0            | 0.789   |
| Weight, kg     | 8.4 ± 1.4      | 8.3 ± 1.6       | 8.1 ± 1.2       | 8.1 ± 1.4       | 0.854   |
| Mothers        |                |                 |                 |                 |         |
| Age, years     | 24.2 ± 4.0     | 27.0 ± 4.5      | 27.4 ± 9.2      | 25.7 ± 5.4      | 0.349   |
| Weight, kg     | 51.6 ± 5.8     | 55.5 ± 8.3      | 55.1 ± 5.8      | 57.9 ± 9.9      | 0.097   |
| Height, cm     | 152.5 ± 6.0    | 152.2 ± 5.0     | 155.4 ± 4.3     | 153.0 ± 6.0     | 0.280   |
| MUAC, mm       | 249.4 ± 17.2   | 260.8 ± 23.2    | 258.6 ± 21.6    | 263.7 ± 22.8    | 0.200   |
| DDMT Weight D₂O (g) | 6.000 ± 0.000 | 10.015 ± 0.081 | 14.989 ± 0.076 | 19.989 ± 0.090 |         |

Data are presented as mean ± SD unless otherwise noted. MUAC=mid-upper arm circumference.
### Table 2. Body composition and breastmilk intake

| Variable               | DDMT-6g (n=19) | DDMT-10g (n=20) | DDMT-15g (n=18) | DDMT-20g (n=18) | P-value |
|------------------------|----------------|-----------------|-----------------|-----------------|---------|
| BMI, kg/m²             | 22.2 ± 2.3     | 24.0 ± 3.8      | 22.8 ± 1.8      | 24.8 ± 4.4      | 0.073   |
| Body fat, kg           | 13.4 ± 4.4     | 19.0 ± 5.9      | 14.6 ± 5.2      | 18.3 ± 7.2      | 0.008   |
| Fat mass, %            | 25.7 ± 7.7     | 33.9 ± 9.3      | 26.1 ± 7.4      | 30.6 ± 6.8      | 0.003   |
| Free fat mass, kg      | 38.2 ± 4.7     | 36.6 ± 6.5      | 40.5 ± 4.6      | 39.7 ± 3.9      | 0.090   |
| Free fat mass, %       | 74.3 ± 7.7     | 66.1 ± 9.3      | 73.9 ± 7.4      | 69.4 ± 6.8      | 0.005   |
| Total body water, kg   | 27.9 ± 3.4     | 26.8 ± 4.8      | 29.7 ± 3.4      | 29.0 ± 2.8      | 0.091   |
| Total body water, %    | 54.4 ± 5.6     | 48.4 ± 6.8      | 54.1 ± 5.4      | 50.8 ± 5.0      | 0.005   |
| Breastmilk intake, g/d | 796.6 ± 292.4  | 739.4 ± 296.3   | 749.8 ± 244.2   | 733.6 ± 207.1   | 0.883   |

*Data are presented as mean ± SD. *ANOVA

## DISCUSSION

The objective of our study was to evaluate whether administering deuterium doses <30 g allows accurate assessment of the human breast milk output in saliva using the new Agilent 4500S for FTIR. Unfortunately, it was not possible to administer the different doses of deuterium to the same lactating mothers because it takes at least 2 months to ensure the total resorption and elimination of deuterium from the body [12,16], though this would have allowed us to compare the different doses using Bland-Altman graphs [24]. We chose an indirect method by selecting lactating mothers living in the same environment and for whom we have data on the human breast milk excretion evaluated by different methods, including DDMT [25,26]. These mothers were then randomly assigned to the four treatment groups. We obtained four similar groups if we consider the age and anthropometric parameters of the lactating mother and the breastfed infant.

The breast milk output values were similar to those reported by Owino in 2011 among lactating mothers selected in the same HZ who had breastfed infants aged 9-10 months [25]. The breastfed infants in the different groups were ≥6 months old, except for one infant who was 3
months old. Therefore, they were already receiving food supplements in addition to breastfeeding. The infants had an average age of 10-11 months with the expected values for this age group. In our opinion, this confirms that the different deuterium doses allowed accurate measurement of the human breast milk output.

With regard to quality control, no particular group recorded invalid curves that could suggest poor diffusion and insufficient resorption of deuterium from mother to child up to day 14. The most plausible hypothesis for these results would be that FTIR with the Agilent 4500S is more sophisticated than previous models and more sensitive in detecting deuterium (15). Nevertheless, these results must be interpreted by taking into account the average weight of the mothers included in the study, which remained <60 Kg even if the maximum values in the different groups were > 60 Kg. The minimum and maximum values observed in the 6 g, 10 g, 15 g, and 20 g groups were 43.5-64.0 Kg, 40.9-75.4 Kg, 44.7-63.7 Kg, and 41.6-78.0 Kg, respectively.

The analysis of human breast milk may require a low dose of deuterium when the mother is low weight and exclusively breastfeeding [27]. Therefore, it is important to conduct similar studies in populations of lactating mothers who are heavier, and with older non-exclusively breastfed-infants. If the findings are confirmed, then the possibility of reducing the conventional 30 g dose and ensuring a good assessment of human breast milk output could be considered.

We observed a difference in body composition between the groups without an effect on breast milk. This supports the theory that excess body fat does not necessarily lead to increased human breast milk. However, our sample size does not allow us to reach a conclusion [28].
CONCLUSION

The different doses of deuterium administered in this study resulted in similar breast milk output. The results suggest that evaluating human breast milk with deuterium doses < 30 g is possible when working with more efficient equipment, such as the Agilent 4500S. Deuterium doses between 6 and 20 g allowed optimal measurement of human breast milk with good sensitivity until day 14. Multicenter studies are needed to confirm the results obtained in DRC.

List of abbreviations:

IAEA: International Atomic Energy Agency
BMI: Body mass index
CRSN: Centre de Recherche en Sciences Naturelles de Lwiro
DDMT: Deuterium dose mother technique
DRC: Democratic Republic of Congo
FFM: Free fat mass
FTIR: Fourier transform infrared spectroscopy
HZ: health zone
LMIC: Low and medium-income country
LPH: Lwiro Pediatric Hospital
MUAC: Mid-upper arm circumference
RIPSEC: Renforcement institutionnel pour les politiques de santé basées sur l’Evidence en République Démocratique du Congo.
TBW: Total body water

WHO: World Health Organization

DECLARATIONS

Ethics approval and consent to participate
The study was authorized by the ethics committee of the Catholic University of Bukavu (UCB/CIES/NC/02B/2019) and by the technical division of the Ministry of Public Health of the DRC (South Kivu Province). All mothers enrolled in the study gave their informed consent. We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable

Availability of data and material
All data generated or analyzed during this study are included in this published article [and it supplementary information files].

Competing interests
The authors declare that they have no competing interests.

Funding
Authors' contributions

CCM, GMB, DBM and GBB produced the original concept of the article, executed the field study (subject recruitment and data collection), and had primary responsibility for the first draft of the manuscript.

AK contributed to data collection and samples analysis in the laboratory which generated curves by the DDMT.

GM and VO validated curves generated by the DDMT and contributed to the enrichment of the manuscript.

MDW and PD validated data analysis, contributed to the intellectual content of the discussion.

All authors read and approved the final manuscript.

Authors’ information

1 Ecole Régionale de Santé Publique, Université Catholique de Bukavu, Bukavu, Democratic Republic of Congo (DRC); 2 Ecole de Santé Publique, Université Libre de Bruxelles, Brussels, Belgium; 3 Centre de Recherche en Sciences Naturelles, Lwiro, DRC; 4 Hôpital Provincial General de Reference de Bukavu, Université Catholique de Bukavu, Bukavu, DRC; 5 National Institute for Scientific and Industrial Research in Zambia (NISIR); 6 International Atomic Energy

Acknowledgements

We would like to thank the IAEA for providing the equipment necessary for carrying out the body composition and breastmilk intake analysis through the Regional Technical Cooperation Project (RAF6052). We also thank the RIPSEC Program for supporting the field survey costs for this study.
REFERENCES

1. Global Nutrition Report group. Global Nutrition Report 2019. https://globalnutritionreport.org/events/nutrition-2019/ 2019

2. FAO, FIDA, OMS, PAM, UNICEF. Annual report: L’état de la sécurité alimentaire et nutrition dans le monde en 2018. Renforcer la résilience face aux changements climatiques pour la sécurité alimentaire et la nutrition. Rome 2018.

3. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M et al. Maternal and Child Nutrition Study Group. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013;382(9890):427-51.

4. Wang H, Bhutta ZA, Coates MM, Coggeshall M, Dandona L, Diallo K et al. Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980–2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1725-74.

5. World Health Organization. Global strategy for infant and young child feeding. 2017. Available from : http://www.who.int/nutrition/topics/global_strategy_iycf/en/.

6. Allen, J.; Hector, D. Benefits of breastfeeding. N. S. W. Public Health Bull. 2005, 16, 42–46. [PubMed]

7. Hellmuth C, Uhl O, Demmelmaier H, Grunewald M, Auricchio R, Castillejo G et al. The impact of human breast milk components on the infant metabolism. PLOS ONE 2018 June 1 (DOI: 10.1371/journal.pone.0197713).
8. Wesolowska A, Sinkiewicz-Darol E, Barbarska O, Bernatowicz-Lojko U, Borszewska-Kornacka MK, van Goudoever JB. Innovative techniques of processing human milk to preserve key components. Nutrients. 2019;11(5):1169.

9. Dror K, Allen LH. Overview of nutrients in human milk. Adv Nutr. 2018;9(Suppl 1):278S–294S.

10. World Health Organization. Quantité et Qualité du lait maternel. Rapport sur une étude collective consacrée à l’allaitement au sein. Genève 1987. https://apps.who.int/iris/bitstream/handle/10665/39082/9242542016.pdf?sequence=1 consulted on November 15, 2020.

11. IAEA. Introduction to the assessment of body composition by deuterium dilution through the analysis of saliva samples by Fourier transform infrared spectroscopy. Human Health Series No. 12. Vienna 2013.

12. IAEA. Technique using a stable isotope to assess breast milk consumption in breastfed babies. Human Health Series No. 7. Vienna 2014.

13. Coward WA, Cole TJ, Sawyer MB, Prentice AM. Breast milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. Hum Nutr Clin Nutr. 1982; 36:141-8.

14. Jennings G, Bluck L, Wright A, Elia M. The use of infrared spectrophotometry for measuring body water spaces. Clin Chem. 1999;45;(7)1077–81.

15. Moore SE, Prentice AM, Coward WA, Wright A, Frongillo EA, Fulford AJC et al. Use of stable-isotope techniques to validate infant feeding practices reported by Bangladeshi women receiving breastfeeding counseling. AJCN. 2007;85(4):1075-82.
16. Rein A, Higgins F. Application of the Agilent 4500 series FTIR to the stable isotope technique for assessing intake of human milk in breastfed infants. Agilent Technologies Inc. December 2, 2013. 5991-3531EN (http://www.agilent.com/chem).

17. Republic Democratic of Congo. Ministère du Plan de la R.D.Congo. Enquête Démographique et de Santé en RDC 2013-2014. Kinshasa 2014.

18. Republic Democratic of Congo. Ministère du Plan de la R.D.Congo. Enquête Démographique et de Santé en RDC 2018-2019. Kinshasa 2019.

19. Matsiko E, Hulshof PJM, van der Velde L, Kenkhaus MF, Tuyisenge L, Melse-Boonstra A. Comparing saliva and urine samples for measuring breastmilk intake with the deuterium oxide dose-to-mother technique among children 2-4 months old. Br J Nutr 2019 Oct 18 (Epub ahead of print; DOI: 10.1017/S0007114519002642).

20. da Costa THM, Haisma H, Wells JCK, Mander AP, Whitehead RG, Bluck LJC. How much human milk do infants consume? Data from 12 countries using a standardized stable isotope methodology. J Nutr. 2010; 140:2227–32.

21. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height, and body mass index-for-age: Methods and development. Geneva. 2006.

22. Owino VO, Kasonka LM, Sinkala MM, Wells JK, Eaton S, Darch T et al. Fortified complementary foods with or without a-amylase treatment increase hemoglobin but do not reduce breast milk intake of 9-mo-old Zambian infants. Am J Clin Nutr. 2007; 86:1094–103.

23. Haisma H, Coward WA, Albernaz E, A Barros A, Victora CG, Wright A et al. 2H2O turnover method as a means to detect bias in estimations of intake of non-breast milk liquids in breast-fed infants. Eur J Clin Nutr. 2005;59(1):93–100.
24. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;327(8476):307–10.

25. Owino VO, Bahwere P, Bisimwa G, Mwangi CM, Collins S. Breast-milk intake of 9-10-mo-old rural infants given a ready-to-use complementary food in South Kivu, Democratic Republic of Congo. Am J Clin Nutr. 2011; 93:1300–4.

26. Donnen P, Brasseur D, Dramaix M, Assimbo V, Hennart P. Effects of cow's milk supplementation on milk output of protein deficient lactating mothers and on their infants’ energy and protein status. Trop Med Int Health. 1997; 2:38-46.

27. Slater C, Kaestel P, Houghton L. Assessing breastfeeding practices objectively using stable isotope techniques. Ann Nutr Metab. 2019; 75:109–113.

28. Diana A, Haszard JJ, Houghton LA, Gibson RS. Breastmilk intake among exclusively breastfed Indonesian infants is negatively associated with maternal fat mass. Eur J Clin Nutr. 2019;73(8):1206-8.
