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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

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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 (D_2O) 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 D_2O instead of 30 g to assess breastmilk 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 March15 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_2O, 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_2O (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 D_2O 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 D_2O 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 chi^2 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 D_20 (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/m^2	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.

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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.

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