SELINUS UNIVERSITY
OF SCIENCES AND LITERATURE

CONTRIBUTION TO IMPROVING THE EVALUATION OF BREASTFEEDING PRACTICE:

APPLICATION OF THE DEUTERIUM OXIDE DOSE-TO-MOTHER TECHNIQUE DURING THE FIRST 6 MONTHS AFTER BABY BIRTH IN 'VALLÉE DU KOU', BURKINA FASO AND COMPARISON OF METHODS OF ASSESSMENT OF EXCLUSIVE BREASTFEEDING.

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DECLARATION

I certify I am the sole author of the thesis. The dissertation entitled " **Contribution to improving the evaluation of breastfeeding practice: Application of the deuterium oxide dose-to-mother technique during the first 6 months after baby birth in 'Vallée du Kou', Burkina Faso and comparison of methods of assessment of breastfeeding"** is my original work. My thesis is written and presented according to the requirements for obtaining the degree of Doctor of Philosophy in Human Nutrition and Nutritional Assessment, at Selinus University. The research articles published and presented in the thesis are collaborative works in which I am the first author.

Nadine Mireille Josepha Danielle COULIBALY

DEDICATION

I dedicate this work to my children: Joel Sidney Stephane, Inamahoro Celia Carmen, Yohannes Strong Stone and Katia Kyria Divine.

"*Soldiers, I wanted to encourage you to never give up whatever your challenges, your mistakes. Love people and do your best to maintain integrity as you have learned from me. The world is changing, but God is still the same*.".

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ABBREVIATIONS

BMI: Body Mass Index
DMT: Dose-to-mother technique
EBF: Exclusive breastfeeding
EFA Essential Faty acid
FFM: Fat free Mass
FM: Fat Mass
FMI: Fat Mass Index
FTIR: Fourier Transform Infra-Red Spectrometry
HM: Human Milk
IAEA: International Atomic Energy Agency
IDA; Iron deficiency anaemia
IFA: Iron, folic acid
IPT: intermittent preventive treatment
IRMS: Isotope Ratio Mass Spectrometry
IYCF: Infant and young children's feeding practice
LAM: Lactational Amenorrhea method
LAZ: length for age Z-score
MUAC: mid-upper arm circumference
NCD: noncommunicable disease
PMTCT: Prevention of Mother to Child Transmission
RDA: Recommended Dietary Allowance
SP: Sulfadoxine and pyrimethamine
SRMSE: Square Root Mean Square Error
TBW: Total body water
UNICEF: United Nations Children's Fund
WAZ: Weight for age Z-score
WHO: World Health Organization
WLZ: Weight for length Z-score

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Abstract

Introduction

Knowledge of infant nutritional status and breastfeeding practice is important for developing a strategy that can help to improve infant nutrition. Exclusive breastfeeding (EBF) for the first 6 months is widely recommended by WHO and UNICEF as feeding practice in early infancy. This also implies the choice of a robust method to ascertain EBF application by mothers. In this work, we describe our contribution to improve assessment of breastfeeding practice using the deuterium oxide dose-to-mother technique in "Vallee du Kou", a rural community of Burkina Faso.

Methods

Forty-six mother-baby pairs were recruited and followed-up to 6 months. We performed breastfeeding assessment through longitudinal follow-up by the means of 4 cross-sectional measurements at 0-1, 2-3, 4-5, and 6-months using the deuterium oxide dose-to-mother technique. We first developed a simple protocol that can help to validate data collected from the studies using the deuterium oxide dose-to-the-mother technique for breastfeeding evaluation. We used the human milk intake calculation spreadsheets of our study (n=180). Based on the original spreadsheets that fitted well with the model (n=87), we established a cut-off of the SRMSE and used it to check and correct the other spreadsheets.

Then, we described exclusive breastfeeding (EBF) practice and the nutritional status of mother-baby pairs from birth up to six months. Infants and mothers' nutritional status was determined by anthropometry and we used the deuterium oxide dose-to-mother technique (DMT) to measure the human milk intake (HM) as well as the non-milk oral water intake (non-HM) by the babies and maternal body composition.

Finally, we compared different methods of assessment of EBF using the McNemar Test.

Results

We found a SRMSE cut-off dependent on the enrichment measured (E_m) by FTIR that was $\mathbf{0.027}\sqrt{\sum(\boldsymbol{E_m})^2}$. We observed within the corrected spreadsheets a significant reduction ($p \leq 0.0001$, n=53) of the SRMSE (90% CI) from 49.78 mg/kg (46.35 mg/kg, 53.20 mg/kg) before correction to 25.88 mg/kg (24.13 mg/kg, 27.64 mg/kg) resulting in a change of HM and non-HM. Therefore, our validation method is very important for optimizing the dose to-mother technique results.

Analysing infant feeding practice, we found that malnutrition was present during all the follow-up, with a high rate of wasting at first month (16.3%), and 6 months (22.7%). The HM intake increase significantly ($p< 0.001$) from 570.0 ± 208.2 g/day at birth month to 848.5 ± 175.6 g/day at 2-3 months and reached the maximum of 923.1 ± 184.2 g/day ($p=0.004$) at 4-5 months when the non-HM was minimal (9.5 ± 67.4 g/day). was optimum at 4 months with 88.89%. But according to the longitudinal evaluation, this rate is reduced significantly ($p<0.001$) to 55.5% at 4 months. So, there was inadequate breastfeeding practice up to 6 months contrary to the engagement of the mothers. We also found the mothers were fat deficient (59-69%) and they suffered from anaemia (49-56%).

Finally, after comparing the different methods of assessment of breastfeeding practice, the results showed that the maternal report overestimated the EBF rate for 20% ($p<0.001$) compared to DMT. When the DMT was considered, there was an overestimation using cross-sectional measurement (36.2%, $p<0.001$) compared to the longitudinal measurement. Based on the WHO model applied to the DMT, we showed the WHO model overestimated the EBF for 26,9% ($p<0.001$) as compared to the DMT longitudinal measurement.

Conclusion

This work contributed to improve the use of the dose-to-mother technique, to provide data on breastfeeding practice in Burkina Faso and reaffirmed the utility of the technique for breastfeeding assessment. The DMT can be used as a routine method for the assessment of EBF during the nutritional surveys or intervention programs.

Keywords: infant nutrition, milk intake, exclusive breastfeeding, deuterium oxide dose-to-mother technique, excel spreadsheet, square root-mean-square error, validation, McNemar test, rural community, Burkina Faso.

CHAPTER 1: INTRODUCTION

1. Background

Infant and young child feeding (IYCF) practices directly affect the health, development and nutritional status of children less than two years of age and, ultimately, impact child survival. Improving IYCF practices in children 0–23 months of age is therefore critical to improved nutrition, health and development [1] as optimal feeding practices are fundamental to a child's survival, growth and development, but too few children benefit.

Measures of child malnutrition are used to track development progress. Malnutrition refers to deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients. The term malnutrition addresses 3 broad groups of conditions: undernutrition, which includes wasting (low weight-for-height), stunting (low height-for-age or low length-for-age) and underweight (low weight-for-age); micronutrient-related malnutrition, which includes micronutrient deficiencies (a lack of important vitamins and minerals) or micronutrient excess; and overweight, obesity and diet-related non-communicable diseases (such as heart disease, stroke, diabetes and some cancers) [2]. Stunting and wasting remain public health problems in low-income countries, where 4·7% of children are simultaneously affected by both, a condition associated with a 4·8-times increase in mortality. Recent evidence shows that stunting and wasting might already be present at birth, and that the incidence of both conditions peaks in the first 6 months of life [3]. Estimates of child malnutrition will help determine whether the world is on track to achieve the Sustainable Development Goals, particularly, target 2.2, to "end all forms of malnutrition by 2030", which falls under goal 2 to "end hunger, achieve food security and improved nutrition, and promote sustainable agriculture" [1].

In the first two years of life, breastfeeding saves lives, shields children from disease, boosts brain development and guarantees children a safe and nutritious food source [4]. The evidence showed no apparent risks in recommending, as a general policy, exclusive breastfeeding for the first six months of life in both developing and developed-country settings [5]. UNICEF and the World Health Organization (WHO) recommend that infants begin breastfeeding within one hour of birth, be exclusively breastfed for the first six months, and continue breastfeeding until 2 years of age or beyond [6, 7]. Exclusive breastfeeding means that the infant receives only breastmilk. No other liquid or solid is given, not even water, except for oral rehydration solution or drops/syrups of

vitamins, minerals or medicines [8]. Assessing objectively this important indicator of infant nutrition will help for better nutrition intervention.

Burkina Faso is a landlocked country in West Africa (Fig.1) with an estimated population of about 21,510,181 inhabitants in 2020 [9]. According to the 2019 human development report, the country ranked 182nd out of 189 with over 40% of its population living below the national poverty line [10]. In that low-income country most of the population lives in rural areas (76% in 2015) with limited resources [11], malnutrition remains one of the most common causes of morbidity and mortality among children under 5 in the country [12]. Since 2009, the country installs a system of control of malnutrition by evaluating infant nutritional status and feeding practice through a national nutrition survey. The surveys showed that the prevalence of stunting among infants of 0-23 months passed from 35.1% in 2009 to 27.3% in 2016; underweight passed from 26% to 19.2% and wasting from 11.3% to 7.6% and exclusive breastfeeding up to 6 months was 16% in 2009 and 55.5% in 2016 [13]. In fact, breastfeeding is common in the country and the national policy on breastfeeding follows the international recommendation which is exclusive breastfeeding (EBF) up to six months, introduction of other foods at 6 months and continuing to breastfeed up to 2 years [5, 7]. EBF is assessed using cross-sectional measurement based on maternal report giving a current status of the infant at the time (24h recall) and during which it is difficult to validate the mothers' declarations on infants' feeding practice. Another limitation of the data produced during breastfeeding evaluation surveys is the fact that up to now there is no data available on the quantity of milk consumed by the babies. So, there is no evidence that infants are fed sufficiently on demand that the breast milk intake by the babies meet their needs (energy and micronutrient). And up to now EBF up to 6 months, based on maternal reports, continues to be low (25% at 4-5 months [14], 40.0% at 0-5.9 months in rural areas [15]). It became important to use new methods that can help to well understand infant feeding practice in the country.

For several years, the amount of breast milk consumed by the babies and water from sources other than breast milk has been assessed using stable isotope technique, more precisely the deuterium dilution method [16-32]. The deuterium is safe [33]; the technique is non-invasive; the procedure does not interfere with the infant's normal feeding pattern. The amount of human milk consumed by the baby over 14 days can be assessed using the deuterium oxide 'dose-to-mother' technique, which involves giving the mother a drink of deuterium labelled water and following the

disappearance of the deuterium from the mother and its appearance in the baby. The technique also allows the baby's intake of water from sources other than human milk and the mother's body composition to be estimated. In the present work, we want to show our contribution to improve the assessment of infant feeding practice at the country level and international nutrition community level. Our work, based on a study we conducted among a rural community of Burkina Faso using the dose-to-mother technique, will be presented through 3 activities:

- Validation of breastfeeding data provided by deuterium oxide dose-to-mother technique
- Assessment of breastfeeding practice and maternal body composition from baby birth up to six months
- Revisiting who exclusive is exclusive breastfeeding practice by comparison of methods of assessment

2. Objectives

2.1. General objective

To use a stable isotope technique to assess infant feeding practice during the first six months after birth.

2.2. Specifics objectives

- To validate breastfeeding data provided by the deuterium oxide dose-to-mother technique
- To find a cut-off for SRMSE calculated in the human- milk spreadsheet
- To assess infant nutritional status from birth up to six months
- To assess milk intake during the first six months of life
- To determine the intake of water from source other than breastmilk from baby birth up to 6 months
- To validate exclusive breastfeeding practice from birth up to 6 months
- To assess lactating mothers' body composition during breastfeeding practice up to 6 months.
- To find the best method that can well assess exclusive breastfeeding practice

Map Sources: UNCS, ESRI.
The boundaries and names shown and the designations used on this map do not imply official endorsement or acceptance by the United Nations. Map created in Sep 2013.

Figure 1: Cartography of Burkina Faso

CHAPTER 2: LITERATURE REVIEW

1. Nutritional status and malnutrition assessment

1.1. Assessment of nutritional status [34]

1.1.1. Introduction

Optimal nutritional status, the state of the body regarding each nutrient and overall body weight and condition, is a powerful factor in promoting health and preventing and treating diseases.

Nutrition assessment includes taking anthropometric measurements and collecting information about a client's medical history, clinical and biochemical characteristics, dietary practices, current treatment, and food security situation.

Health care providers assess clients' nutritional status for many reasons:

- To identify people at risk of malnutrition for early intervention or referral before they become malnourished
- To identify malnourished clients for treatment -malnourished people who are not treated early have longer hospital stays, slower recovery from infection and complications, and higher morbidity and mortality.
- To track child growth.
- To identify medical complications that affect the body's ability to digest food and utilize nutrients

1.1.2. Anthropometry

Anthropometry is the measurement of the size, weight, and proportions of the body. Common anthropometric measurements include weight, height, MUAC, head circumference, and skinfold.

Body mass index (BMI) and weight-for-height are anthropometric measurements presented as indexes. Each of these indexes is recorded as a z-score. Z-scores are measured in standard deviations (SD), and describe how far and in what direction an individual's anthropometric measurement deviates from the measurement for a healthy person of the same age and sex (median)

1.1.3. Biochemical assessment

Biochemical assessment means checking levels of nutrients in a person's blood, urine, or stools. Lab test results can give trained medical professionals useful information about medical problems that may affect appetite or nutritional status.

1.1.4. Clinical assessment

Clinical assessment includes checking for visible signs of nutritional deficiencies such as bilateral pitting oedema, emaciation (a sign of wasting, which is a loss of muscle and fat tissue as a result of low energy intake and/or nutrient loss from infection), hair loss, and changes in hair colour. It also includes taking a medical history to identify comorbidities with nutritional implications, opportunistic infections, other medical complications, usage of medications with nutrition-related side effects, food and drug interactions, and risk factors for disease (e.g., smoking, alcohol use, overweight) that affect or are affected by diet and nutritional status [35].

1.1.5. Dietary assessment

Assessing food and fluid intake is an essential part of nutrition assessment. It provides information on dietary quantity and quality, changes in appetite, food allergies and intolerance, and reasons for inadequate food intake during or after illness. The results are compared with recommended intake such as recommended dietary allowance (RDA) to counsel clients on how to improve their diets to prevent malnutrition or treat conditions affected by food intake and nutritional status (e.g., cardiovascular disease, cancer, obesity, diabetes, and hyperlipidaemia). Common ways to assess dietary intake are 24h-recall, food frequency questionnaire, food group questionnaire.

1.1.6. Food security assessment

Food security means having, at all times, both physical and economic access to sufficient food to meet dietary needs for a productive and healthy life [36].

This definition includes food availability (sufficient quantities of food available consistently to all people in a country, region, or household through domestic production, imports, and/or food assistance), food access (adequate resources to obtain a sufficient quantity and quality of food), and food utilization/consumption (proper biological use of food by the body) [34].

A family is food secure when its members do not live in hunger or fear of hunger. Food insecurity is often rooted in poverty and has long-term impacts on the ability of families, communities and countries to develop and prosper. Prolonged undernourishment stunts growth, slows cognitive development and increases susceptibility to illness [36].

1.2. Malnutrition [2]

1.2.1. Definition

Malnutrition refers to deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients. The term malnutrition addresses 3 broad groups of conditions:

- Undernutrition, which includes wasting (low weight-for-height), stunting (low height-for-age) and underweight (low weight-for-age);
- Micronutrient-related malnutrition, which includes micronutrient deficiencies (a lack of important vitamins and minerals) or micronutrient excess; and
- overweight, obesity and diet-related noncommunicable diseases (such as heart disease, stroke, diabetes and some cancers).

1.2.2. Undernutrition

There are 4 broad sub-forms of undernutrition: wasting, stunting, underweight, and deficiencies in vitamins and minerals. Undernutrition makes children in particular much more vulnerable to disease and death.

Low weight-for-height is known as wasting. It usually indicates recent and severe weight loss, because a person has not had enough food to eat and/or they have had an infectious disease, such as diarrhoea, which has caused them to lose weight. A young child who is moderately or severely wasted has an increased risk of death, but treatment is possible.

Low height-for-age is known as stunting. It is the result of chronic or recurrent undernutrition, usually associated with poor socioeconomic conditions, poor maternal health and nutrition, frequent illness, and/or inappropriate infant and young child feeding and care in early life. Stunting holds children back from reaching their physical and cognitive potential.

Children with low weight-for-age are known as underweight. A child who is underweight may be stunted, wasted, or both.

1.2.3. Micronutrient deficiency

Inadequacies in intake of vitamins and minerals, often referred to as micronutrients, can also be grouped together. Micronutrients enable the body to produce enzymes, hormones, and other substances that are essential for proper growth and development.

Iodine, vitamin A, and iron are the most important in global public health terms; their deficiency represents a major threat to the health and development of populations worldwide, particularly children and pregnant women in low-income countries.

1.2.4. Overweight

Overweight and obesity are when a person is too heavy for his or her height. Abnormal or excessive fat accumulation can impair health. Overweight and obesity result from an imbalance between energy consumed (too much) and energy expended (too little). Globally, people are consuming foods and drinks that are more energy-dense (high in sugars and fats), and engaging in less physical activity.

Body mass index (BMI) is an index of weight-for-height commonly used to classify overweight and obesity. It is defined as a person's weight in kilograms divided by the square of his/her height in meters (kg/m^2). In adults, overweight is defined as a BMI of 25 or more, whereas obesity is a BMI of 30 or more.

1.2.5. Diet-related noncommunicable diseases

Diet-related noncommunicable diseases (NCDs) include cardiovascular diseases (such as heart attacks and stroke, and often linked with high blood pressure), certain cancers, and diabetes. Unhealthy diets and poor nutrition are among the top risk factors for these diseases globally.

1.2.6. Situation of malnutrition worldwide

In 2014, approximately 462 million adults worldwide were underweighted, while 1.9 billion were either overweight or obese. In 2016, an estimated 155 million children under the age of 5 years were suffering from stunting, while 41 million were overweight or obese. Globally, in 2020, 149 million children under 5 were estimated to be stunted (too short for age), 45 million were estimated to be wasted (too thin for height), and 38.9 million were overweight or obese.

2. Maternal nutrition [37]

2.1. Nutrition during pregnancy

2.1.1. Control of Maternal Iron Deficiency Anaemia

a. Iron/Folic Acid or multiple micronutrient supplementation:

- All pregnant women should receive 30 Iron/Folic Acid tablets a month for six months (180 tablets in total). Health workers should provide enough Iron/Folic Acid tablets to last until the next foreseen ante-natal care.
- All pregnant women should be counselled on side effects, compliance and safety of IFA supplements.
- All pregnant women should be counselled on the importance of adhering with daily intake of one tablet of Iron/Folic Acid (daily intake of one IFA tablet - 60 mg iron + 400 µg folic acid) for six months.
- All pregnant women should be screened for pallor on every visit. Pregnant women with pallor should receive Iron/Folic Acid supplementation according to IDA treatment protocol.

b. Adequate micro-nutrient intake

All women should be counselled on how to increase iron-intake through locally available iron-rich sources, including combining foods that help absorption and avoiding foods that hinder absorption. Low-income pregnant or lactating women who cannot access the minimum required diet should be supported by means of fortified food supplementation and sprinkles.

c. De-worming in endemic areas

All pregnant women should receive a single dose of Albendazole (400 mg) or a single dose of Mebendazole (500 mg) in the second trimester (4th - 6th month). If hookworms are highly endemic (prevalence over 50%), pregnant women should be given a second dose in the third trimester (7th - 9th month). All pregnant women should be advised on preventive measures (sanitation and foot-wear).

d. Malaria control in endemic areas

All pregnant women should receive 2 doses of IPT:

- ✓ First dose: 3 tablets SP once during the 4th to 6th months of pregnancy.
- ✓ Second dose: 3 tablets SP once during the 7th to 9th months of pregnancy.

All pregnant and breastfeeding women should be promptly treated for clinical infections

All pregnant and breastfeeding women should be counselled on how to use the Insecticide Treated Net (ITN)

e. Adequate food-intake

All pregnant women should be counselled by a trained service provider on:

- Increased energy intake through one additional meal a day.
- Improved variety (cereal/starchy roots plus animal foods/legumes/nuts plus fruit/vegetable).
- Reduced workload (or at least have regular resting moments).
- Daily use of iodized salt for all family members.
- Monitor weight gain in pregnancy (a woman should gain 10-12 kg weight during pregnancy)

a. Get ready for breastfeeding

All pregnant women should be counselled by a trained service provider on:

- Initiation of breastfeeding within 60 minutes from birth.
- Importance of colostrum or "First Milk".
- Feeding only breast milk, no water or other liquids/foods for the first six months.
- Prevention of Mother to Child Transmission of HIV/AIDS (PMTCT)

All HIV negative and unknown-status pregnant women should be encouraged to go for HIV Individual counselling and Testing.

All HIV positive pregnant women should be counselled by a trained service provider on infant feeding in the context of HIV.

2.2. Nutrition during breastfeeding:

2.2.1. Control of Maternal Iron Deficiency Anaemia

Breastfeeding women should continue to receive IFA supplementation in the first three months postpartum where the prevalence of anaemia is equal to or more than 40%. Where prevalence of anaemia is less than 40%, only breastfeeding women who did not receive the recommended amount

during pregnancy should be provided with IFA supplementation in the first three-six months postpartum.

2.2.2. Adequate food-intake

All breastfeeding mothers should be counselled by a trained service provider on:

- Increased energy intake through two additional meals a day (a lactating mother requires 550 calories extra per day).
- Improved variety (cereal/starchy roots plus animal foods/legumes/nuts plus fruit/vegetable).
- Reduced workload (or at least have regular resting moments).
- Daily use of iodized salt for all family members.
- Continue to breastfeed during common illnesses and pregnancy.

2.2.3. Birth spacing

All breastfeeding mothers should be clearly advised by a trained service provider on the correct use of the Lactational Amenorrhea Method (LAM) as a family planning method based on the simultaneous existence of all conditions below:

- Lactating mother did not get menses;
- Baby is exclusively and frequently breastfed;
- Baby is less than six months old.
- Prescribed contraceptives by health workers should not have side effects on breastfeeding

3. Infant and young children's feeding and nutrition

3.1. Importance

Infant and young child feeding (IYCF) practices directly affect the health, development and nutritional status of children less than two years of age and, ultimately, impact child survival. Improving IYCF practices in children 0–23 months of age is therefore critical to improved nutrition, health and development [1].

3.2. General recommendation

What, when and how young children are fed during the first two years of life lay the foundation for survival, growth and development. Ideally, infants should be put to breast within one hour of birth, breastfed exclusively for the first 6 months of life and continue to be breastfed up to 2 years of age and beyond. Starting at 6 months, breastfeeding should be combined with safe, age-appropriate feeding of nutritious solid, semi-solid and soft foods.

3.3. Indicators for assessing IYCF

In 2021, UNICEF and WHO published a set of updated indicators for assessing infant and young child feeding practices during this critical window of birth to up to 2 years of age. There are 17 indicators (6 for breastfeeding, 9 for complementary feeding and 2 others indicators). The table 1 gives the breastfeeding indicators.

Table 1: Breastfeeding indicators

Indicator	Short name	Age group	Definition
1 Ever breastfed	EvBF	Children born in the last 24 months	Percentage of children born in the last 24 months who were ever breastfed
2 Early initiation of breastfeeding	EIBF	Children born in the last 24 months	Percentage of children born in the last 24 months who were put to the breast within one hour of birth
3 Exclusively breastfed for the first two days after birth	EBF2D	Children born in the last 24 months	Percentage of children born in the last 24 months who were fed exclusively with breast milk for the first two days after birth
4 Exclusive breastfeeding under six months	EBF	Infants 0–5 months of age	Percentage of infants of 0–5 months of age who were fed exclusively with breast milk during the previous day
5 Mixed milks feeding under six months	MixMF	Infants 0–5 months of age	Percentage of infants of 0–5 months of age who were fed formula and/or animal milk in addition to breast milk during the previous day
6 Continued breastfeeding 12–23 months	CBF	Children 12–23 months of age	Percentage of children 12–23 months of age who were fed breast milk during the previous day

3.4. Infant malnutrition

3.4.1. Classification

The different forms of malnutrition are determined according to the WHO classification (table2)

Table 2: World Health Organization (WHO) classification of nutritional status of infants and children [38]

Nutritional status	Age: birth to 5 years Indicator and cut-off value compared to the median of the *WHO child growth standards*[a]
Obese	Weight-for-length/height[b] or BMI-for-age >3 standard deviations (SD) of the median
Overweight	Weight-for-length/height[b] or BMI-for-age >2 SD and ≤3 SD of the median
Moderately underweight	Weight-for-age <−2 SD and ≥−3 SD of the median
Severely underweight	Weight-for-age <−3 SD of the median
Moderate acute malnutrition	Weight-for-length/height[b] or BMI-for-age ≤−2 SD and ≥−3 SD of the median, or mid-upper arm circumference ≥115 mm and <125 mm
Severe acute malnutrition	Weight-for-length/height[b] or BMI-for-age <− 3 SD of the median or mid-upper arm circumference <115 mm, or bilateral pitting oedema
Moderately stunted (moderate chronic malnutrition)	Length/height-for-age[b] ≤−2 SD and ≥−3 SD of the median
Severely stunted (severe chronic malnutrition)	Length/height-for-age[b] <−3 SD of the median
Moderately wasted	Weight-for-length/height ≤−2 SD and ≥−3 SD of the median
Severely wasted	Weight-for-length/height <−3 SD of the median

[a]WHO child growth standard: methods and development. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. Geneva: World Health Organization; 2006 (http://www.who.int/nutrition/publications /childgrowthstandards_technical_report_1/en/).

[b]Weight-for-length used in infants and young children aged 0–23 months and weight-for-height used for children aged 24 months and older.

3.4.2. Low birth weight [39]

Birthweight is the first weight of the newborn obtained after birth. For live births, birthweight should preferably be measured within the first hour of life, before significant post-natal weight loss has occurred. Low birthweight is defined as less than 2,500 grams (up to and including 2,499 grams).

3.4.3. Prevalence of infants and young children's malnutrition worldwide

Nearly half of all deaths in children under 5 are attributable to undernutrition [2, 40]. These mostly occur in low- and middle-income countries. At the same time, in these same countries, rates of childhood overweight and obesity are rising.[2]. Undernutrition puts children at greater risk of dying from common infections, increases the frequency and severity of such infections, and delays recovery [40]. In 2014, approximately 462 million adults worldwide were underweighted, while 1.9 billion were either overweight or obese [2].

In 2015, 20.5 million new-borns, an estimated 14.6 percent of all babies born globally that year, suffered from low birthweight [39]. These babies were more likely to die during their first month of life and those who survived face lifelong consequences, including a higher risk of stunted growth [41], lower IQ [42] and adult-onset chronic conditions such as obesity and diabetes [43].

In 2016, an estimated 155 million children under the age of 5 years were suffering from stunting, while 41 million were overweight or obese. Globally, in 2020, 149 million children under 5 were estimated to be stunted (too short for age), 45 million were estimated to be wasted (too thin for height), and 38.9 million were overweight or obese [2].

The figure 2 gives prevalence of infant malnutrition in 2000 and 2020.

3.4.4. Prevalence of infants and young children's malnutrition in Burkina Faso

Since 2009, the country installs a system of control of malnutrition by evaluating infant nutritional status and feeding practice through a national nutrition survey. The surveys showed that the prevalence of stunting among infants of 0-23 months passed from 35.1% in 2009 to 27.3% in 2016; underweight passed from 26% to 19.2% and wasting from 11.3% to 7.6% [13]. In 2020; the prevalence of acute malnutrition, chronic malnutrition and underweight at the national level were respectively 9.1% (including 1.0% in the severe form); 24.9% and 17.6%. Overweight affected 2.8% of children, including 1.9% of children suffering from obesity [44].

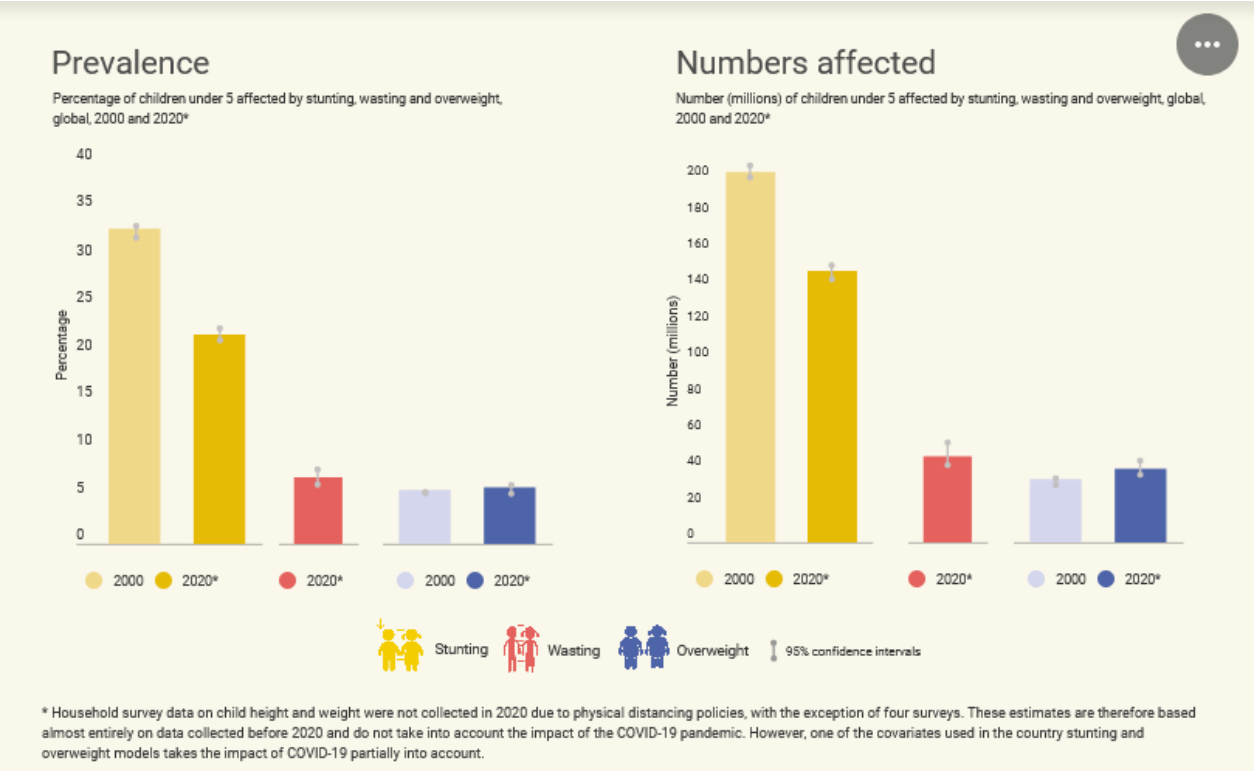


Figure 2: Prevalence of infant malnutrition in 2000 and 2020,

Source: UNICEF/WHO/World Bank Joint Child Malnutrition Estimates, 2021 Edition. retrieved from ref [40]

4. Breastfeeding

4.1. Lactation

4.1.1. Physiology [45]

Lactation is secretion and yielding of milk by females after giving birth. The milk is produced by the mammary glands, which are contained within the breasts.

The breasts, unlike most of the other organs, continue to increase in size after childbirth. Although mammary growth begins during pregnancy under the influence of ovarian and placental hormones, and some milk is formed, copious milk secretion sets in only after delivery. Since lactation ensues after a premature birth, it would appear that milk production is held back during pregnancy. The mechanism by which this inhibitory effect is brought about, or by which lactation is initiated at delivery, has long been the subject of an argument that revolves around the opposing actions of estrogen, progesterone, and prolactin, as studied in laboratory animals, goats, and cattle. During pregnancy, the combination of estrogen and progesterone circulating in the blood appears to inhibit milk secretion by blocking the release of prolactin from the pituitary gland and by making the mammary gland cells unresponsive to this pituitary hormone. The blockade is removed at the end of pregnancy by the expulsion of the placenta and the loss of its supply of hormones, as well as by the decline in hormone production by the ovaries, while sufficient estrogen remains in circulation to promote the secretion of prolactin by the pituitary gland and to favorite lactation.

4.1.2. Human Milk composition at different stage

Milk can be regarded as an emulsion of fat globules in a colloidal solution of protein, together with other substances in a true solution. Two constituents of milk-the protein casein and milk sugar, or lactose-are not found elsewhere in the body.

The milk released from the breast when lactation starts differs in composition from the mature milk produced when lactation is well established. The early milk, or colostrum, is rich in essential amino acids, the protein building blocks essential for growth; it also contains the proteins that convey immunity to some infections from mother to young, although not in such quantity as among domestic animals. The human infant gains this type of immunity largely within the uterus by transferring these antibody proteins through the placenta; the young baby seldom falls victim to mumps, measles, diphtheria, or scarlet fever. For a short time after birth, proteins can be absorbed from the intestine without digestion, so that the acquisition of further immunity is facilitated. The

growth of harmful viruses and bacteria in the intestines is probably inhibited by immune factors in human milk. After childbirth, the composition of milk gradually changes; within four or five days, the colostrum has become transitional milk, and mature milk is secreted some 14 days after delivery. Some variations between human colostrum, transitional milk, and mature milk and cow's milk are shown in table 3.

Table 3: Some constituents of human colostrum, transitional, and mature milk and of cow's milk (average values per 100 millilitres whole milk)

	colostrum (1–5 days)	**transitional (6–14 days)**	**mature (after 14 days)**	**cow's milk**
energy, kcal*	58	74	71	69
total solids, g	12.8	13.6	12.4	12.7
fat, g	2.9	3.6	3.8	3.7
lactose, g	5.3	6.6	7.0	4.8
protein, g	2.7	1.6	1.2	3.3
casein, g	1.2	0.7	0.4	2.8
ash, g	0.33	0.24	0.21	0.72
calcium, mg	31	34	33	125
magnesium, mg	4	4	4	12
potassium, mg	74	64	55	138
sodium, mg	48	29	15	58
iron, mg	0.09	0.04	0.15	0.10

*Kilocalorie; sufficient energy to raise the temperature of 1 kilogram of water 1 degree centigrade.

4.2. Breastfeeding practice

4.2.1. Importance

The offer of the mother's breast to her baby is a biologically and ethically unquestionable right of both mother and child and is of fundamental importance for the survival and quality of life of the nursing baby during its first years of life [46].

Breastfeeding is the normal way of providing young infants with the nutrients they need for healthy growth and development. It is considered as the most single effective way of saving the lives of millions of young children, and a deeply rooted and valued practice in many societies, especially those of developing [47].

4.2.2. Benefit for infants

Breastfeeding is the natural, efficient way of using the readily available reserve for feeding babies and unequalled to no other way of providing ideal nutrition for the healthy growth and development of infants [48, 49].

Breastfeeding is particularly advantageous because of the nutritional, immunologic, and psychological benefits. Human breast milk is superior to modified cow's milk formulas, which may lack essential and beneficial components and are not absorbed as easily or as quickly by the infant. Maternal breast milk provides vitamins, minerals, protein, and anti-infectious factors; antibodies that protect the infant's gastrointestinal tract are supplied, resulting in a lower rate of enteric infection in breast-fed than in bottle-fed babies. The bonding that is established through breast-feeding is advantageous to building the parent-child relationship [45].

In addition to improving child survival and protecting against life-threatening and chronic illnesses, breastfeeding promotes healthy growth and boosts early child development. Breastfeeding supports healthy brain development and is associated with higher performance in intelligence tests among children and adolescents across all income levels [50].

4.2.3. Benefits for the mother

Breastfeeding is not just good for the babies; it is good for mothers as well. Indeed, breastfeeding has been shown to protect against post-partum haemorrhage, postpartum depression, ovarian and breast cancer, heart disease and type 2 diabetes [51]. It is estimated that improving breastfeeding rates could prevent an additional 20,000 maternal deaths from breast cancer [49]. The benefits of

breastfeeding are well documented. The table 4 inspired by the review of Del Ciampo and Del Ciampo [46] summarises the different effects of breastfeeding on maternal health.

Table 4: Benefits of Breastfeeding

Immediate	Long-term
Uterine involution and reduced bleeding [52] Reduced infection Lactational amenorrhea [49, 51, 53] Reduced adiposity and weight, improved body image [54, 55] Reduced postpartum depression [56, 57] Reduced stress and anxiety [58]	**Reduced:** cancer (breast, ovarian, endometrium)[41, 59-63] Endometriosis [64], diabetes [65, 66], osteoporosis [67] , blood pressure and cardiovascular diseases [68-70], metabolic syndrome [71], rheumatoid arthritis [72], Alzheimer's disease [73] and multiple sclerosis[74]

4.2.4. Promoting breastfeeding practice

Many factors contribute to creating a positive environment for breastfeeding. At the national level, policies guaranteeing parental leave and the right to breastfeed in the workplace are critical, as are restrictions on the marketing of breastmilk substitutes. Within health facilities, mothers need information and support to breastfeed immediately after birth, and beyond. Positive social norms that support and encourage breastfeeding, including in public spaces, serve to empower mothers to breastfeed [50]. In many African countries, because of the important contribution of breastfeeding to the child's optimal growth, and the unavailability of the alternative formula milk or its cost

implications, it is crucial to recognize that efforts by healthcare professionals that are geared towards its successful implementation with emphasis on the cultural importance and the social pressure to breastfeed [75, 76]. In communities, support from trained counsellors and peers, including other mothers and family members, plays a key role. The support of men, husbands and partners cannot be underestimated. Interventions are most effective when they are implemented in combination, rather than piecemeal or in isolation [50].

Indeed, studies have found that combined implementation of pro-breastfeeding interventions within health systems and the community has the potential to increase exclusive breastfeeding rates by 2.5 times [77]. In countries like India and Viet Nam [78, 79], governments have been successful in protecting breastfeeding through implementation of supportive policies guaranteeing six months' paid maternity leave, and have put in place strong legislation regulating the marketing of breastmilk substitutes, bottles and teats [80].

In countries such as Sri Lanka and Turkmenistan, the promotion of baby-friendly hospitals, which comply with the Ten Steps to Successful Breastfeeding, has helped to increase rates of breastfeeding. Almost 90 percent of women in Turkmenistan, and almost all mothers in Sri Lanka give birth in hospitals certified as baby-friendly [81] and both of the countries have nearly universal rates of breastfeeding [82].

4.2.5. Exclusive breastfeeding practice

Exclusive breastfeeding means that the infant receives only breast milk. No other liquids or solids are given–not even water–with the exception of oral rehydration solution, or drops/syrups of vitamins, minerals or medicines [83].

WHO Global Strategy for Infant and Young Child Feeding recommends that infants be exclusively breastfed until they turn six months of age [84]. Exclusive breastfeeding is the safest and healthiest option for children everywhere, guaranteeing infants a food source that is uniquely adapted to their needs while also being safe, clean, healthy and accessible. Evidence suggests that infants in low- and middle-income countries who received mixed feeding (foods and liquids in addition to breast milk) before six months were nearly three times more likely to die than those who were exclusively breastfed [85]. Exclusive breastfeeding protects against diarrhoea, lower respiratory infections, acute otitis media and childhood overweight and obesity [49].

5. Stable isotope technique to assess breastfeeding practice [86]

5.1. Introduction

The descriptions given in this part of the review of literature are based on the IAEA handbook that describes the use of stable isotope technique to assess the intake of human milk by the breastfed infant [86].

In many countries, although breastfeeding is widespread, only a small proportion of infants are exclusively breastfed and only limited information is available about the quantities of human milk consumed and the time of introduction of other foods into infants' diets. This lack of information is due, at least partly, to the difficulties involved in measuring the intake of human milk.

The conventional technique to measure the intake of milk is to weigh infants before and after each feed. This is known as 'test weighing'. The technique is time-consuming and the procedure can disturb the normal feeding pattern [87]. In many settings, infants are nursed frequently, on demand, including during the night, which results in practical limitations to the use of test weighing.

The practical problems associated with test weighing can be overcome by using the stable isotope technique. The amount of human milk consumed by the baby over a period of 14 days can be assessed using the deuterium oxide 'dose-to-mother' technique (Fig.3), which involves giving the mother a drink of deuterium labelled water and following the disappearance of the deuterium from the mother and its appearance in the baby (Fig.4). The technique also allows the baby's intake of water from sources other than human milk and the mother's body composition to be estimated [17, 20, 28]. For example, the deuterium oxide dose-to-mother technique can be used to:

- evaluate the efficacy of counselling and education programmes on infant feeding practices [27, 31];
- evaluate the association between the intake of human milk by breastfed infants and maternal body composition [29] ;
- evaluate community nutrition programmes for lactating women [25] ;
- evaluate the effect of the introduction of complementary foods on human milk intake by breastfed babies and
- quantify nutrient flux or transfer of toxic elements from mother to baby [26, 86].

Deuterium is a stable (non-radioactive) isotope of hydrogen with the symbol 2H. It is given orally as deuterium oxide (2H2O) and, after mixing with body water, is eliminated from the body in urine, saliva, sweat and human milk.

Deuterium oxide is metabolized in the body in the same way as water and is dispersed through the body water within a matter of hours. Body water can be sampled in the form of saliva, urine, plasma or human milk and the enrichment of deuterium can be measured by isotope ratio mass spectrometry (IRMS) or Fourier transform infrared spectrometry (FTIR). FTIR is not suitable for analysis of urine or human milk samples. The technique is not as sensitive as IRMS and, therefore, a larger dose of deuterium oxide is required. However, FTIR instrumentation is easier to use and maintain than that of IRMS, is less expensive to buy and the cost of analysis is lower; FTIR is, therefore, particularly suitable in settings where resources are limited [86].

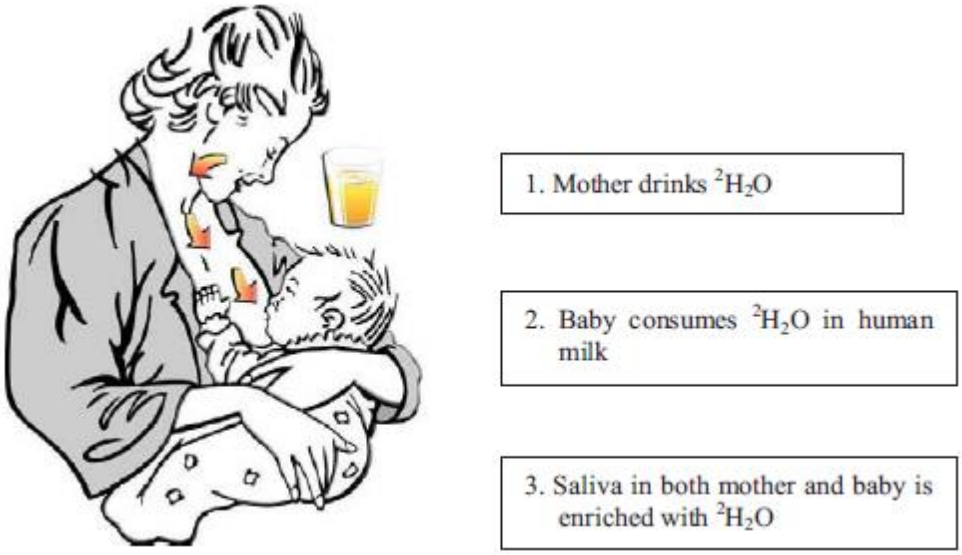


Figure 3: the dose-to-mother technique

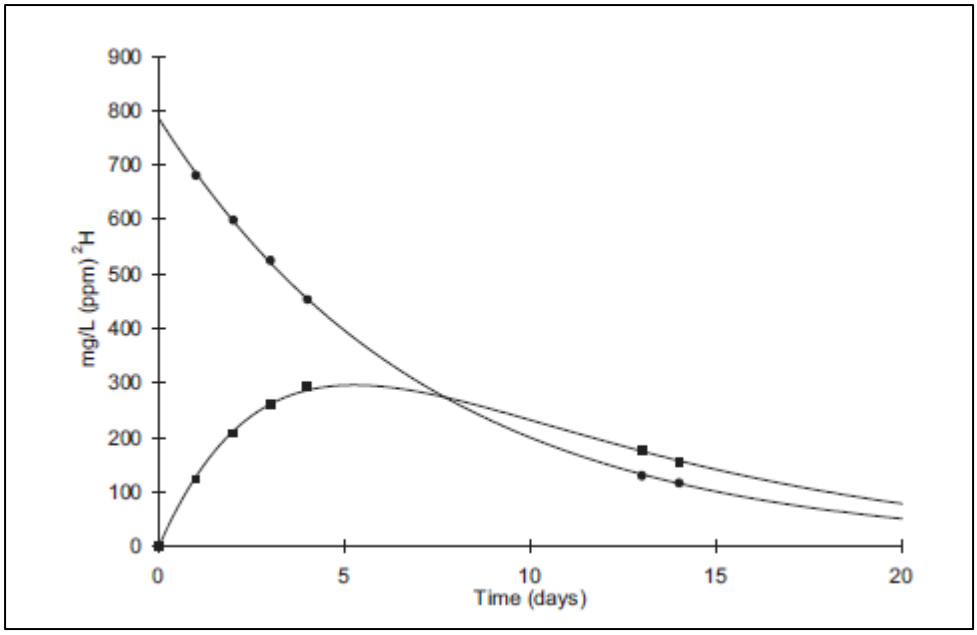


Figure 4: the disappearance of the deuterium from the mother and its appearance in the baby

5.2. The dose-to-mother technique for assessing human milk intake and lactating mother's body composition

5.2.1. The two compartments steady-state model of water flow in a mother-baby pair

The deuterium oxide dose-to-mother technique was first described by A. Coward and co-workers in 1982 [17]. Assessment of human milk intake and intake of water from sources other than human milk is based on a two compartments steady-state model [88]. In the two-compartment model, the mother's body water (Vm) is the first compartment and the baby's body water (Vb) is the second compartment. These two compartments are connected by the flow of milk from the mother to the baby (Fbm). In a steady-state model, the total water input is equal to the total water output. In figure 5, F signifies a flow of water.

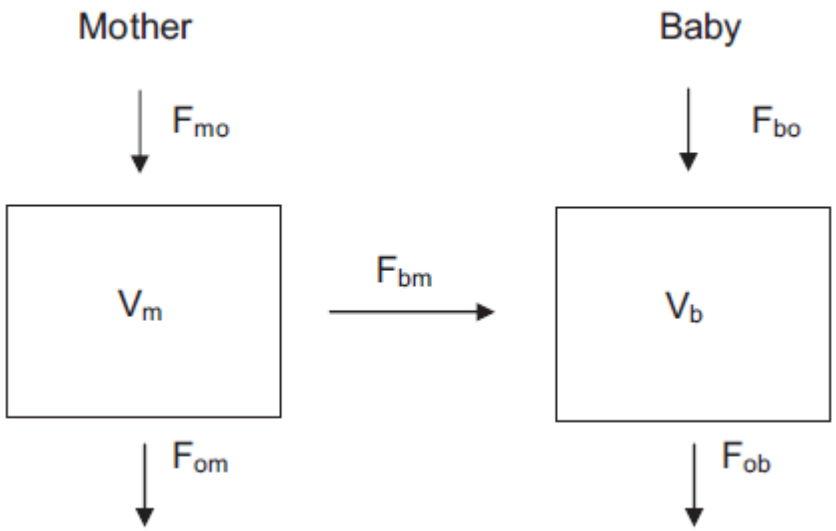


Figure 5: Two compartment steady-state model of water flow in a mother–baby pair.

F = flow; m = mother; b = baby; o = outside; V = volume TBW; Vm = mother's TBW volume; Vb = baby's TBW volume; Fmo = from outside to mother; Fbo = from outside to baby (non-breast fluid intake); Fbm = from mother to baby (breast milk intake); Fom = from mother to outside; Fob = from baby to outside.

5.2.2. Assumptions of the model

The two compartments steady-state model is based on a number of assumptions. Where these assumptions do not hold true, an adjustment is included in the calculations.

The assumptions are:

- The body water pool in both the mother and the baby is a single compartment in each individual.
- The dose of deuterium equilibrates rapidly and uniformly throughout the body water pool of the mother and her baby.
- The size of the body water pool in the mother is constant. The baby's body water pool is assumed to change linearly with time due to growth.
- All water, regardless of the route of exit, is labelled with deuterium in proportion to deuterium in the body water pool.
- Deuterium leaves the system only as water.

– Water intake by the baby is only by ingestion.

The deuterium dose is in the form of deuterium labelled water, also referred to as deuterium oxide (2H2O or D2O).

5.2.3. Validity of the assumptions

– Assumption 1: The body water pool in both the mother and the baby is a single compartment in each. This is true.
– Assumption 2: The deuterium dose equilibrates rapidly and uniformly throughout the body water pool of the mother and her baby. This is true. The deuterium dose is fully equilibrated.
– Assumption 3: The size of the body water pool in the mother is constant, but the baby's body water pool changes linearly with time. In weight stable healthy adults, water input is equal to water output over a two weeks period. If the mother's weight changes, it could be due to changes in body fat, which does not affect the body water pool size, or changes in FFM, which will affect the body water pool size. The baby will grow during the two weeks assessment period; therefore, the baby's TBW will increase. An adjustment must be made for the change in size of the baby's TBW pool. The mother's TBW at the baseline is measured by isotope dilution, but when the deuterium oxide dose is given to the mother, it is not possible to measure the baby's TBW unless a second stable isotope (e.g. ^{18}O) is given to the baby. This would complicate the procedure and make it much more expensive. In addition, ^{18}O requires mass spectrometry for analysis. The baby's TBW is predicted from its body weight (W) using the formula of Wells [15]: $TBW = 0.84\ W^{0.82.}$
– Assumption 4: All water, regardless of the route of exit, is labelled with deuterium in proportion to deuterium in the body water pool. This is not true. An adjustment has to be made for isotopic fractionation in water lost from the baby's body as water vapour in breath and by transdermal evaporation.
– Assumption 5: Deuterium leaves the system only as water. This is not strictly speaking true. A small amount of deuterium exchanges with hydrogen atoms (mainly in proteins) in both the mother's and the baby's body. This process is known as non-aqueous exchange. The error introduced by predicting rather than measuring TBW in the baby is greater than non-aqueous exchange; therefore, for the purposes of assessing human milk intake, non-aqueous exchange is ignored.

– Assumption 6: Water intake by the baby is only by ingestion. Atmospheric water can be absorbed through the skin and the lungs of the baby, with alveolar exchange being the largest component. A correction is necessary to take account of this non-oral water intake, which is estimated to be 6.3% of total water intake.

5.3. Calculation of human milk intake (M) and intake of water from sources other than human milk (Fs) in the baby

5.3.1. Base of the calculation

Intake of human milk and water from sources other than human milk can be calculated by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby. An example is illustrated in Figure 6.

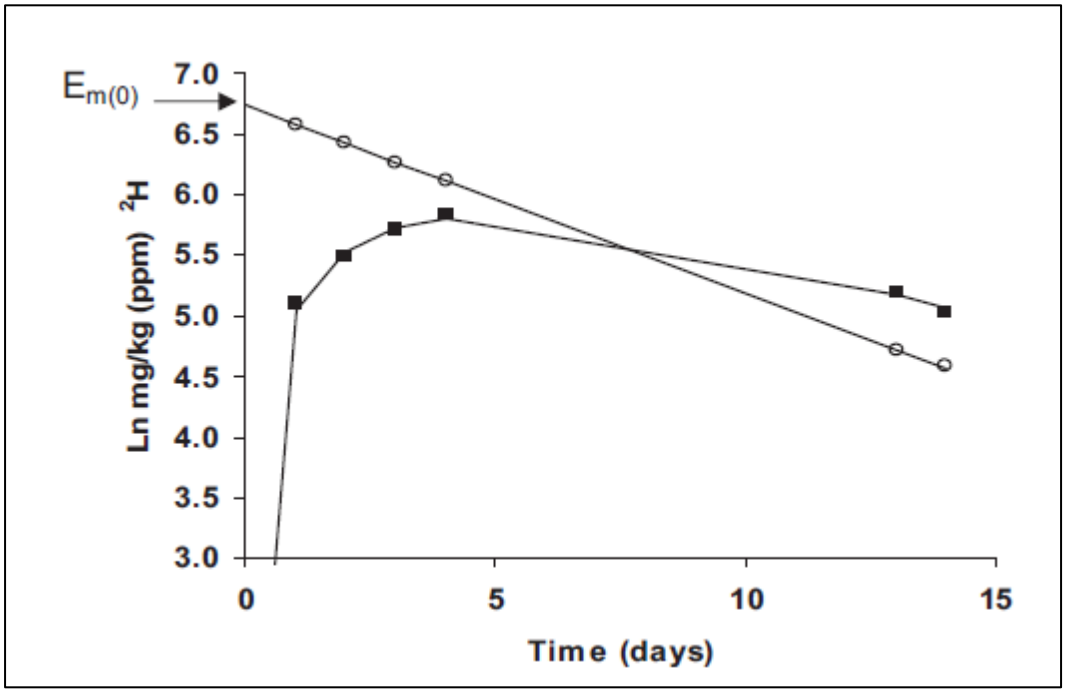


Figure 6: Deuterium enrichment in the body water of a mother (○) and her baby (■).

In the steady state, water turnover in the mother is given by a single exponential equation:

Equation 1

$$\frac{E_{m(t)}}{E_{m(0)}} = e^{-k_{mm}t}$$

where

Em(t) is the deuterium enrichment in the mother's body water at time t, in mg/kg or ppm;

t is the time since the dose was taken, ie. time post-dose in days;

Em(0) is the deuterium enrichment in the mother's body water at time zero mg/kg (ppm), i.e. the y intercept of the isotope elimination curve (log/linear plot of enrichment of 2H in body water versus time) (see Fig.6);

kmm is the fractional water turnover in the mother (kg/d), i.e., the gradient of the isotope elimination curve (see Fig.6).

Data from the baby are fitted to the following multi-exponential model:

Equation 2

$$E_{b(t)} = E_{m(0)}\left(\frac{F_{bm}}{V_b}\right)\left(\frac{e^{-k_{mm}t} - e^{-(F_{bb}/V_b)t}}{(F_{bb}/V_b) - k_{mm}}\right)$$

where,

Eb(t) is the deuterium enrichment in the baby's body water at time, t, in mg/kg (ppm);

t is the time since the dose was taken by the mother, i.e. time post-dose in days;

Em(0) is the deuterium enrichment in the mother's body water at time zero mg/kg (ppm), i.e. the y intercept of the mother's isotope elimination curve (log/linear plot of enrichment of 2H in the mother's body water versus time) (see Fig.6);

Fbm is the transfer of water from the mother to the baby via human milk (kg/d);

Vb is the baby's total 2H distribution space (kg). Vb is assumed to change linearly with initial and final values determined from the baby's weight (W, kg). $Vb = 0.84\ W^{0.82}$ [89];

kmm is the fractional water turnover in the mother (kg/d), i.e., the gradient of the mother's isotope elimination curve (see Fig.6);

Fbb is the total water loss in the baby (kg/d). Curve fitting can be performed using the 'Solver' function in Microsoft Excel.

5.3.2. Calculation of M: human milk intake by the baby

Human milk intake by the baby is calculated from the flow of water from the mother to the baby, assuming that human milk is 87.1% water [90].

M = Fbm/0.871 kg/d Measured human milk intake is often expressed as g/d.

5.3.3. Calculation of Fs: the baby's intake of water from sources other than human milk

a. Bases of the calculation

The baby's total intake of water includes water from the oxidation of milk solids (protein, fat and carbohydrate) and water from sources other than human milk. The total water input derived from human milk is Fm. Calculation of Fs assumes that water input equals water output. Allowance must be made for the baby's growth (Fg) and for an increase in TBW during the two weeks of saliva sampling and the fact that water lost in the baby's breath and by transdermal evaporation (Fob) is subject to isotopic fractionation (see Appendix III for more information on isotopic fractionation), and for absorption of atmospheric water by the skin, mainly in the lungs (Fa). Water input = (Fm + Fa + Fs).

Water input (Fm + Fa + Fs) equals water output plus water from growth

(Fob + Fg); therefore: Fs = Fob + Fg – Fm – Fa

b. Calculation of total water input to the baby derived from human milk (Fm)

The flow of water from the mother to the baby (Fbm) represents free water in milk and does not include water from the oxidation of milk solids (protein, fat and carbohydrate):

Human milk is assumed to contain 87.1% water, 1.3% protein, 4.1% fat and 7.2% carbohydrate [90].

The yield of water from 1 g of protein is 0.41 g, from 1 g of fat1.07 g and from 1 g of carbohydrate 0.55 g.

Therefore, oxidation of milk solids gives about 9 g of water per 100 g of human milk.

Total water input to the baby derived from human milk (Fm) is given by: $Fm = Fbm + 0.09M$

c. Adjustment for the baby's growth (Fg)

Growth of the baby during the experimental period will result in a small change in the baby's deuterium distribution space, which is related to its TBW, and in this context is known as Vb. Vb is assumed to change linearly with initial and final values determined from the baby's weight (W, kg). $Vb = 0.84\ W^{0.82}$ [89]. Water gained during the experimental period, Fg, is given by:

$Fg = (Vb, day14 – Vb, day0)/14$

d. Adjustment for isotopic fractionation (Fob)

Deuterium is lost from body water via breath and insensible routes via the skin (transdermal evaporation) more slowly than hydrogen, for the reasons described above; therefore, Fbb must be corrected for isotopic fractionation. Total water output from the baby, i.e., flow from the baby to the outside (Fob), which includes water lost as urine, sweat, in faeces and in breath, includes a correction for isotopic fractionation. The isotopic fractionation factor for deuterium between water vapour and water liquid is 0.946 at 37ºC. It is assumed that 85% of the baby's water output is not fractionated and that the remaining 15% is fractionated by a factor of 0.946. Thus, the correction factor is $0.85 + (0.946 \times 0.15) = 0.9919$.

Fob is given by: $Fob = Fbb/0.9919$

e. Adjustment for water absorbed by the skin (Fa)

For non-oral water intake in the infant (Fa), a correction factor is necessary for environmental water influx to the baby, which is composed of atmospheric water absorbed through the skin and the lungs. Alveolar exchange is the largest component. Non-oral water intake is estimated as 6.3% of total water intake[23]. As total water intake is equal to total water output, Fa is given by:

$$Fa = 0.063(Fob + Fg)$$

f. Calculation of oral water intake from sources other than human milk (Fs)

$$Fs = Fob + Fg – Fm – Fa$$

There is an error associated with the estimate of the baby's intake of water from sources other than human milk, because of the assumptions made in this calculation. This error (25 ± 62 ml/day) results in a small apparent intake of water from sources other than human milk in babies who are truly exclusively breastfed [31]. Finally, a work has been done recently to determine the cut-off for exclusive breastfeeding validation that is 86.6 g/day of water from sources other than milk [91].

5.4. Calculation of maternal body composition

The mother's body composition is estimated from her TBW, which is measured by deuterium dilution. The calculations assume that the body is composed of fat and fat-free mass (FFM). Fat mass (FM) is the difference between body weight and FFM. FFM can be estimated from TBW as follows. The volume of TBW is slightly less than the volume of distribution of the deuterium dose because some of the deuterium is sequestered in non-aqueous substances (mainly proteins) by a process known as non-aqueous exchange. VD is the volume of distribution of deuterium (also known as the pool space). When the natural logarithm of deuterium enrichment in the mother's body water is plotted against time, the distribution is a straight line. VD is calculated from the y intercept of the linear regression line through the data (Fig. 6). The value of the y intercept is given the notation, Em(0) (enrichment of 2H in the mother's body water at time zero):

$$VD\ (kg) = \text{deuterium oxide dose (mg)}/(Em(0))$$

VD must be corrected for non-aqueous isotopic exchange. Non-aqueous isotopic exchange for deuterium is assumed to be 4.1% of the pool space. TBW is, therefore, calculated by dividing VD by 1.041: $TBW\ (kg) = VD/1.041$.

The hydration of FFM in the body is remarkably constant between species, but is higher in infants than in adults. Here, we are only concerned with the mother. FFM in adults is assumed to be 73.2% water. This is known as the hydration of FFM. Thus: FFM (kg) = TBW (kg)/0.732.

FM is calculated as the difference between FFM and body weight:

FM (kg) = body weight (kg) – FFM (kg)

FM% = FM (kg)/body weight (kg) × 10

CHAPTER 3: MATERIALS AND METHODS

1. Study design and subjects

1.1. Design

The study was conducted in "Vallée du Kou", a rural area in Western Burkina, at 25 Km in the west of Bobo, Dioulasso (Fig. 1). Forty-six mother-baby pairs were recruited in 2 steps. A cohort of 24 pairs was firstly recruited and followed up to 12 months, then a second cohort of 22 pairs was included. We collected field data from August 2008 to January 2011. In our work, we describe the first 6 months follow-up. It was a longitudinal descriptive study with 4 cross-sectional measurements at birth month, 2-3 months, 4-5 months and at 6 months. At each visits anthropometry measurement were done, haemoglobin level was taken in mother and Human milk intake and lactating woman body composition were assessed using the "deuterium oxide-dose-to-mother" technique (DMT).

1.2. Subject

The mother-baby pairs were recruited at the health centre no later than 2 weeks after baby birth. The mothers had already received breastfeeding counselling at inclusion, as during antenatal visits. They agreed to exclusively breastfeed their babies and stay in the area during all the follow-up and they did not have twin to breastfed due to the model of calculation used in the technique (the two compartments model where the mother is one compartment and the baby another compartments) [86]. The exclusion criteria were pregnancy and leaving the area during the follow-up. The study received the approval of the Institutional Ethical committee (CEICM-0201/2008) and the mothers signed an informed consent form before they could take part.

2. Validation of deuterium oxide dose-to- mother data

2.1. Introduction

2.1.1. Context

This work was motivated by the necessity of finding how the result produced after breastmilk calculation are enough valid to be taken into account as a database for statistical analysis. Effectively, after breastmilk calculation we remarked that some sheet gave low SRMSE while in the others it was very high and all the value didn't fit well the model giving some data point out of the deuterium kinetic curve. So, we tried to find a cut-off of the SRMSE and to correct the data in order to optimize our results and finally to share the methodology with the end user of the dose-to-

mother technique. Finally, the work has been published in 2019 as an article in a peer-reviewed journal [92].

2.1.2. Justification

The deuterium oxide dose-to-mother technique [17] is presented as a promise tool for exclusive breastfeeding (EBF) evaluation. The dose is orally administrated to the mother and saliva samples are collected over a period of 14 days. Deuterium enrichment in saliva is analyzed by infrared mass spectrometer (IRMS) or Fourier transformed infrared spectrometer (FTIR). The IRMS is more sensitive than the FTIR, but the FTIR is improved to be a simple, rapid method for measuring deuterium enrichment in physiological fluid [86, 93] and it is appropriated for limited resource setting as it permit overcoming the practical problem link to the IRMS equipment, which is more expensive and need specialist for analysis [86, 94].

The calculation of human milk (HM) intake and intake of water from sources other than milk (non HM) is performed by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby [86]. The quality of the output (HM and non-HM) will be as high as the mean square error between observed and fitted enrichment is low.

Ideally, the Square Root Mean Square Error (SRMSE) that characterizes the best fitting should be zero, but this is impossible in practice. Moreover, there is no range of values set for the SRMSE. That represents a big challenge in the data validation as it is difficult to know if the outputs of the calculation (HM and non-HM) are really of high quality and should be used for the final results. Therefore, it becomes necessary to have a cut-off that can be used for the validation of the data.

So, we developed a prediction method of the SRMSE that could help to check and correct or removed inappropriate data if necessary. The aim was to validate our own data and finally to provide the user of the technique a guideline that could help them to well assess their results and decide on their validity.

2.2. The materials: The Human Milk calculation spreadsheets

The HM intake estimation using deuterium dilution is performed by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby and it lies on complex formulas [23, 88, 90, 95, 96].

Standard Excel spreadsheets (Fig.7) were developed by Cowards and coworkers [17, 28] to facilitate this estimation. A template is given in IAEA website. The users are invited to enter the mother and the baby's age, their anthropometrical measures and different deuterium enrichment values of sample collected at different time points on both mother and child. The spreadsheet uses prepared equations in order to estimate the model parameters using the Excel's solver add-on. These parameters are then used to compute body composition and milk intake information. This is a very good way to simplify advanced techniques for simple using, such as using z-score tables.

We worked on 180 spreadsheets. Each spreadsheet represented a mother-baby dyad data collected during our breastfeeding evaluation study that we conducted through 4 cross-sectional measurements from baby birth up to 6 months. Briefly, after anthropometric measurement and saliva sample collection from the mother and her baby, an accurate dose of 30g of deuterium oxide (DO) was given to the mother. The mother fed the baby as usual and the baby received the deuterium from his mother through breast milk. Post doses saliva samples were collected from the mother and her baby at day 1, 2, 3, 4, 13 and 14 post dosing. Deuterium enrichment in the saliva was analyzed by FTIR (Shimadzu 8400S) according to the method described in the IAEA manual [86]. The data were entered into the human milk calculation spreadsheet and the solver function was used to perform the calculation by minimizing the SRMSE.

Instructions

THIS SPREADSHEET IS FOR USE WITH FTIR DATA ONLY.
DO NOT CUT AND PASTE DATA: IT CORRUPTS THE FORMULAE. COPY AND PASTE MAY BE USED FOR DATES
1) Rename spreadsheet: Human milk calcs "Study ID"
2) Enter data in cells with blue background
3) Enter the time (hr:min) and date (day/month/year) the dose was taken by the mother in cells B25 and B26
3) If SOLVER is not already installed on your computer, click on TOOLS, ADD-INS. Select "Solver Add-in"
4) Go to cell J61 (the Target Cell)
5) Click on TOOL, SOLVER
6) The Solver parameters window opens, click on "Solve".
This will determine the constants in cells F55:F58 which give the line of best fit to the mother's and baby's data
7) The main outputs are human milk intake (J55) and intake of water from sources other than human milk (J60).
8) The mother's body composition is in cells B57:B59

Participant Study ID	**20**				
MOTHER'S DATA			**BABY'S DATA**		
Date of Birth			Date of Birth	28-août-08	
age	33	years	sex	M	
weight	72,45	kg	age		months
			start weight	6,05	kg
DOSE DATA			final weight	6,50	kg
Bottle number	20				
Weight D_2O (g) in bottle	30,03				
Date dose taken by mother	05-nov-08				
Time dose taken by mother	09:43				

(1) Data for mothers saliva

Date	time	time since dose (days)	Deuterium enrichment ppm (mg/kg)	ppm (mg/kg)	mean	SD	CV (%)	ppm (mg/kg) calc	mean sq error MSE
		0						792,0	
06-nov-08	09:04	0,97	666	664	665,0	1,29	0,19	667,9	8,81
07-nov-08	09:06	1,97	570	573	571,7	1,83	0,32	560,5	124,63
08-nov-08	09:29	2,99	457	463	460,0	3,94	0,86	469,2	83,33
09-nov-08	07:04	3,89	401	399	400,2	1,36	0,34	400,8	0,39
18-nov-08	09:12	12,98	81	80	81,0	0,66	0,82	81,6	0,42
19-nov-08	09:54	14,01	71	75	72,7	2,70	3,71	68,2	21,05
								sum =	238,63

(2) Data for baby's saliva

Date	time	time since dose (days)	Deuterium enrichment ppm (mg/kg)	ppm (mg/kg)	mean	SD	CV (%)	Body Water	ppm calc	mean sq error MSE
		0						3,68	0,0	
06-nov-08	09:25	0,99	120	119	119,6	0,13	0,11	3,69	132,2	159,33
07-nov-08	09:47	2,00	224	216	219,7	5,75	2,62	3,71	215,3	19,66
08-nov-08	09:56	3,01	267	267	267,0	5,06	1,89	3,72	260,6	41,41
09-nov-08	07:42	3,92	273	280	276,9	1,24	0,45	3,74	279,5	6,68
18-nov-08	09:20	12,98	139	138	138,6	0,11	0,08	3,88	142,0	11,88
19-nov-08	10:16	14,02	127	126	126,5	0,11	0,09	3,90	124,4	4,01
									sum =	242,98

Subject	20										
MOTHER'S BODY COMPOSITION				**KINETIC DATA**							
D space (V_m), kg	37,9	% body wt	normal values	Em(0) =	791,98	ppm		Human milk intake (M)	**0,884**	kg.day^{-1}	**884**	ml.day^{-1}
TBW, kg	36,4	50,3	40-65%	k(mm) =	0,18	day^{-1}		Water input from milk (Fm)	0,850	kg.day^{-1}		
Fat-Free Mass, kg	49,8	68,7	50-85%	F(bb) =	0,93	day^{-1}		Water used in growth (Fg)	0,016	kg.day^{-1}		
Body fat, kg	22,7	31,3	10-50%	F(bm) =	0,77	kg.day^{-1}		Total water output (Fob)	0,939	kg.day^{-1}		
Fat, %	31,3			k(bm) =	0,02	day^{-1}		Non-oral water intake (Fa)	0,060	kg.day^{-1}		
								Non-milk oral intake (Fs)	**0,045**	kg.day^{-1}	**45**	ml.day^{-1}
								Square root MSE (mg/kg)	21,95	Total error		

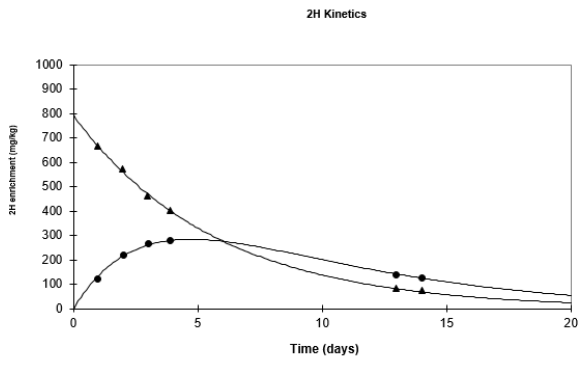


Figure 7: the human milk intake spreadsheet template

2.3. Classification of the spreadsheet

The best fitting with the model requires complete data for each mother-baby dyad, including weight, the 12 post-doses enrichment and the time of saliva samples collection. And all the measured enrichment must be on the kinetic curve.

In the absence of an objective reference method to assess data quality, the goodness of fitted curves provided by the models was observed. We assumed that the modeling curve, which is very close to the actual data, should come from valid data. Finally, that method was used as the first approach to determine reference spreadsheets.

We checked all the spreadsheets for data completeness and we separated them into 2 groups: complete spreadsheets and incomplete spreadsheets. In each group of spreadsheets, we identified those without unfitting data. That meant that all the enrichment seemed to be on the deuterium kinetic curve. All spreadsheets with unfitted data (one or several enrichments are out of the curve) have been subjected to the correction. With the complete fitted spreadsheets, we calculated the cut-off and used it to check the others data.

2.4. Determination of the SRMSE cut-off

Our approach was based on the hypothesis that even if there is an error between the measured and calculated enrichment, this error should be proportional to the measured enrichment for both mother and baby data during the 14 days. This supposes that the measurement error would be smaller with low measures and larger with high values.

So, the best fitting with the model would signify that there is a unique number α such as

$$|E_c - E_m| = \alpha E_m \quad (1)$$

With $E_c = \text{enrichment calculated}$ and $E_m = \text{enrichment measured}$.

By definition, the mean square error (MSE) between the measured and the calculated enrichment at each day is:

$$MSE = (E_c - E_m)^2 \quad (2)$$

For each mother-baby dyad during the 14 days, the sum of MSE would be:

$$\sum(E_c - E_m)^2 = \sum(\alpha E_m)^2 \quad (3)$$

Then the square root-mean-square error should be

$$SRMSE = \alpha\sqrt{\sum(E_m)^2} \quad (4)$$

After determining α value for each fitting spreadsheet, we excluded from the group the spreadsheets with α value higher than mean +2SD as outlier. We recalculated the mean + 2SD as α_{max}.

We assumed that the experimental SRMSE should be less than $\alpha_{max}\sqrt{\sum(E_m)^2}$ before the spreadsheet could be validated.

2.5. Correction of the unfitted data.

The correction consisted of removing the enrichment line that didn't fit with the model. We assumed that if there were incomplete spreadsheets with remaining data that fitted well with the model; it is also possible to remove the unfitted data in order to have the best fitting and generate results with small error. Therefore, the corrected file will seem to be an incomplete spreadsheet with data fitting well with the model.

The enrichment that presented a big MSE were removed if the SRMSE was greater than $\alpha_{max}\sqrt{\sum(E_m)^2}$.

For the mother, as deuterium kinetic in mother is exponential in the model, the maternal body composition is determined by back extrapolation method using the logarithm that is linear, so three well-fitting enrichment data are sufficient to make the maternal curve.

For the baby, as the enrichment increases from day 1 and reach the maximum at day 4 and decrease to day 14, we assume that it is important to avoid removing 2 adjacent data in order to maintain this kinetic and the day 14 can't be removed as it is crucial to HM intake calculation in the model. So, with these criteria, no more than 50% of the enrichment data should be removed.

2.6. Final statistic.

After removing the enrichment line, the SRMSE was refined and then it was compared with the cut- off. All the spreadsheets that couldn't be corrected for these criteria were rejected. All the data were recorded in an Excel spreadsheet and analyzed with STATA software. The proportions of the different type of spreadsheets were calculated. We analyzed the effect of the correction of enrichment on the SRMSE as well as on the HM and the non-HM by mean comparison before and

after correction. Mean comparison was made using the t-test (paired t-test and one sample mean comparison test).

3. Assessment of breastfeeding practice

3.1. Follow-up and DMT application

3.1.1. General procedure

After anthropometric measurement and baseline saliva sample collection from the mother and her baby at day 0, an accurate dose of 30g of deuterium oxide (DO) was given to the mother. The mother fed the baby as usual and the baby received the deuterium from his mother through breast milk. We collected saliva samples from the mother and her baby at day 1, 2, 3, 4, 13 and 14 post dosing. We performed day 0 and 14 activities in the health centre and we collected saliva samples from day 1 to day 13 at participants' homes or working place.

3.1.2. Anthropometry measurement

The mothers were weighted with light clothing nearest 0.1 kg with an electronic scale (SECA model 813.) and heighten nearest 0.1 cm at day 0 with a stadiometer (SECA model 203).

The babies were weighted naked with a precision of 10g using an electronic scale (SECA 383) and their length was measured with an infantometer (SECA 417) to 0.1 cm at day 0 and 14.

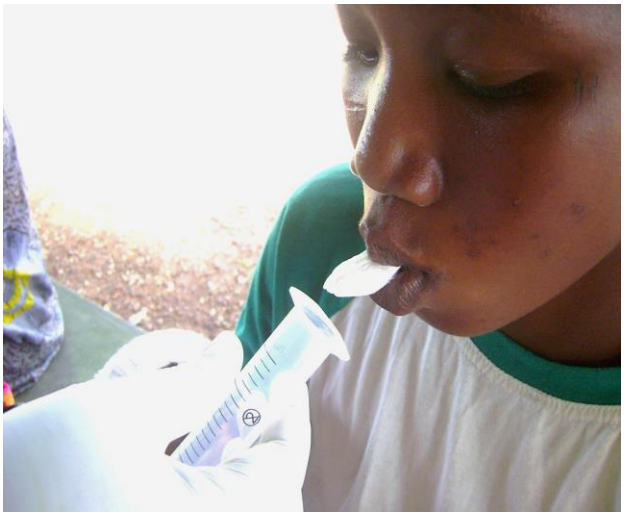
3.1.3. Deuterium oxide (DO) administration

The DO was administrated to the mother after ensuring that baseline saliva samples were collected from her and her baby. Then an accurate dose of 30g of labelled DO (Deuterium oxide99.99% atom D, Sigma-Aldrich) prepared in 60ml propylene bottle and weighted near 0.001g was given to the mother to drink with a straw. The dose was followed by 20 ml of drinking water added twice to the bottle and shaken before the mother consumed in order to ensure that the entire DO was absorbed.

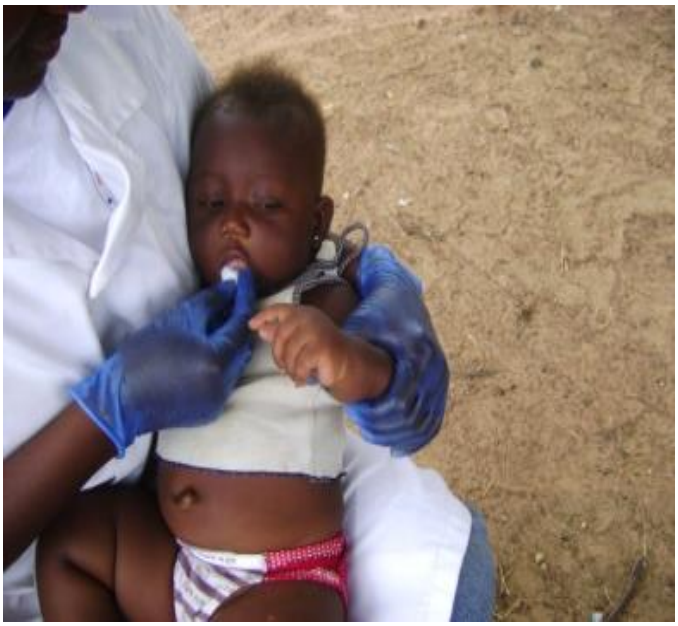
Pictography 1: Deuterium oxide dose consumption by the mother

3.1.4. Saliva sample collection

After ensuring that she has not eaten or drunk anything for at least 30 minutes before collection, saliva was collected from the mother using a sterile cotton wool ball to soak up saliva. The cotton ball was transferred directly from her mouth into a 20 ml syringe and saliva was pressed in a 5ml vial (NUNC 5ml cryo-tube with internal screw caps). Sample collection in the baby was performed by putting the sterile cotton balls in the hollow of the cheeks. The baby wasn't leaved from the eyes during the procedure to avoid that he could swallow the cotton. The balls were removed and transferred into a 10ml syringe to be pressed into a tube and 2ml was collected. Food preparation gloves (vinyl or nitrile) were used for the procedure. Daily samples were transported from the field in electric cool box (4°C) to be frozen at -20°C in IRSS-DRO laboratory, Bobo-Dioulasso, Burkina Faso.



Pictography 2: Saliva sample collection in mother



Pictography 3: Saliva sample collection in infant

3.1.5. Maternal haemoglobin measurement

The haemoglobin concentration was measured in the mother capillary blood in duplicated at day 0 with the HemoCue machine (HemoCue 201 Sweden).

3.1.6. Laboratory analysis

Deuterium enrichment in the saliva samples was analysed twice by Fourier Transformed Infrared Spectrometer (FTIR 8400S, Shimadzu) in the IRSS-DRO laboratory according to the standard operating procedure described in the AIEA handbook [86]. A standard prepared in the laboratory and validated by calibration curve was used as a reference for the analysis. The deuterium enrichment was determined by comparing the sample spectrum to the standard spectrum using the "Isotope" software MRC-NHR.

Pictography 4: FTIR 8400S instrument

3.2. Data management and statistical analysis

3.2.1. Infants' nutritional status

The babies' growth indicators (weight for length Z-score (WLZ), length for age Z-score (LAZ), and weight for age Z-score (WAZ) were calculated using WHO Anthro V3.1.0 software according to WHO growth standard [97]. Any Z<-2 was identified as malnutrition and Z<-3 as severe malnutrition.

3.2.2. HM intake and EBF assessment

The calculation of breastmilk intake (HM), oral water intake from sources other than milk (non-BM) was performed by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby using standard excel spreadsheets [17, 86]. The sum of the squares of

difference between observed and fitted values for mother and baby data combined (the MSE) was minimized using solver function.

Following the method of Coulibaly et al. [92] that we described in the section 2 (2.1 to 2.6) of the materials and methods , the calculation was validated if: $SRMSE \leq 0.027\sqrt{\sum(Em)^2}$ (5), where *Em* is the enrichment measured by FTIR.

Exclusive breastfeeding was assessed for non-BM ≤ 86.6 g/day according to Li et Al. study [91]

3.2.3. Maternal nutritional status

Haemoglobin (Hb) concentration was designed as anaemia among mother for any Hb <12g/l [98].

The body mass index (BMI) of the mothers was calculated from height and weight as BMI=Weight (kg)/Height $(m)^2$ and the maternal nutritional status was determined according to WHO body mass index (BMI) classification in table 5 [99].

Table 5: BMI classification

BMI	Nutritional status
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Pre-obesity
30.0–34.9	Obesity class I
35.0–39.9	Obesity class II
Above 40	Obesity class III

While calculating the HM intake, the spreadsheet gives at the same time the maternal body composition as total body water (TBW), fat free-mass (FFM) and fat mass (FM) [86]. After the

spreadsheet validation, the maternal body composition is validated following the method of Slater and Preston [100] by comparing the TBW to the predicted one as:

TBW = 7.4×Height^3 for 18.5<BMI<29.9,

TBW = 6.5×Height^3 for BMI<18.5

TBW = 8.7×Height^3 for BMI>29.9

The maternal body composition was validated based on the method of Slater and Preston [100] by comparing the total body water (TBW) calculated from the model to the predicted TBW given according to BMI range, as followed

Then the fat mass index (FMI) was determined as:

$$FMI\ (kg.m^{-2}) = FM\ /height^2 \qquad (7)$$

Finally, fat deficit and over fat were determined from the FMI according to the classification of Kelly, Wilson and Heymsfield [101].

3.2.4. Final statistics

The end data were entered with Excel 2016 (Microsoft Office, USA) and analysed with STATA 13 (Statacorp Texas, USA). Descriptive statistic was developed. Means were compared using a t-test (paired and unpaired) and proportions were compared using the z-test. Any difference was statistically significant for the p-value < 0.05.

4. Revisiting how exclusive is exclusive breastfeeding

4.1. Introduction

Exclusive breastfeeding means that the infant receives only breastmilk. No other liquid or solid is given, not even water, except for oral rehydration solution or drops/syrups of vitamins, minerals or medicines [8]. Assessing objectively this important indicator of infant nutrition will help for better nutrition intervention.

EBF is usually measured using the 24h recall. This indicator is defined as the percentage of infants of 0-5 months of age who received only breast milk during the previous day among the number of infants 0–5 months of age in the community [1, 102, 103]. This is a survey indicator recommended by the WHO for EBF assessment.

Different studies showed that the 24h recall gives only a current status at a determined age and overestimates the EBF rate comparatively to recall since birth or repeated recall for seven days [104-106]. All these methods (24h recall, recall since birth, repeated recall for seven) are based on maternal or caregiver reports and are subjected to social desirability bias.

The deuterium oxide dose-to-mother technique [17] has been used to validate maternal report on exclusive breastfeeding and showed that there was a misreporting using 24 hours recall as a method of assessment of EBF practice with an important discrepancy between maternal report and the biological result [28, 30-32, 107-109].

In this part of our work, we analysed the evolution of exclusive breastfeeding practice as recommended by WHO by comparing different techniques (maternal report and dose-to-mother technique (DMT)) and methods of assessment (longitudinal and cross-sectional): the maternal report (24h recall), the DMT cross-sectional giving the current status at an age, the DMT cross-sectional by cumulating different ages and the DMT longitudinal since birth. The aim was to find the most accurate method to assess EBF that is closed to the exclusive breastfeeding definition according to the WHO recommendation.

4.2. Original study and data management

From the 46 mother-baby pairs of the original dataset, only the pairs with complete breastfeeding data at all the four successive evaluations (0-1 month, 2-3 months or 4-5 months and 6 months) were considered for the final analysis, meaning the DMT data at each time point were available and validated [92].

4.3. Statistical analysis

Data were analysed with Stata 13 (Stata Corporation Texas, USA). Descriptive statistics were first developed to calculate means and proportions. Means were compared using t-test. We used the McNemar's test (classical and exact test) to compare the exclusive breastfeeding rate provided by four different methods of evaluation (table 6). The McNemar test, also called matched case control test (MCC) can easily compare the result of two tests applied to the same population and has been already used to compare two methods of assessment of breastfeeding practice [106]. In this work, the MR was first compared to the DMT cross-sectional assessment. After that, the DMT-cross-sectional assessment was compared to the DMT-longitudinal. We identified two types of cross-sectional assessment: the cross-sectional assessment giving the current status (DMT-CS) at each

specific age (the 0-5 months is disaggregated to 0-1month, 2-3 months and 4-5 months) and the cross-sectional assessment accumulating data of different age from birth to under 6 months (DMT-AA). We limited the comparisons to 0-5 months' age range in order to adapt the calculation to the WHO indicator of EBF. The choice between the chi-square p value and the exact binomial p value to make a decision after a McNemar test decision depended on the sum of the discordant pair in the contingency table. Based on Pembury-Smith and Ruxton guidance, an asymptotic chi-square approach was considered if the number of discordant is more than 25, otherwise, this number is <25, the exact p value was chosen [110]. The type 1 error was fixed at 0.05.

Then after, the infants were classified by feeding mode and the one-way ANOVA was used to compare the BM intake of the different group of feeding at each age, including 6 months of age.

Table 6: Methods for EBF assessment and calculation of the indicators

Methods	Definition	Calculation of EBF rate under 6 months
MR (24h)	The infant received only breastmilk the previous 24h.	(Infants 0-5 months of age who received only breast milk during the previous day)/Infants 0-5 months of age) x 100
DMT-CS	The non-breastmilk oral water intake ≤ 86.6 g/day at a specific age	(Infants of a specific age with non-BM ≤ 86.6 g/day) /Infants of that specific age) x 100
DMT-CA	The non-breastmilk oral water intake ≤ 86.6 g/day at least once during the period under 6 months.	(Infants 0-5 months of age with non-BM ≤ 86.6 g/day)/Infants 0-5 months of age) x 100
DMT-LA	The non-breastmilk oral water intake is ≤ 86.6 g/day in each period under 6 months	(Same infants with non-BM ≤ 86.6 g/day at each age from birth to 5 months)/ Infants of 5 months of age) x 100

MR= Maternal Report, DMT-CS: Dose-to-Mother Technique for a Current Status, DMT-CA: Dose-to-Mother Technique by Cumulating Ages, DMT-LA: Dose-to-Mother Technique Longitudinal Assessment

CHAPTER 4: RESULTS

1. Validation of data provided by deuterium oxide dose-to mother technique

1.1. Final value of the SRMSE cut-off and the validated spreadsheets.

As shown in table 7, the mean SRMSE (95% CI) of the complete spreadsheets was 23.37 mg.kg^{-1} (22.01 mg.kg^{-1}, 24.73 mg.kg^{-1}) and the mean $\boldsymbol{\alpha}$ (95% CI) was 0.017 (0.015, 0.018) with α_{max}= 0.027. The SRMSE cut-off is given by the equation 3

Table 7: SRMSE and α value of the fitted sheets (n=87)

	Mean	95 %CI		Max
SRMSE (mg.kg^{-1})	23.37	22.01	24.73	38.96
α value	0.017	0.015	0.018	0.027

Equation 3: SRMSE max value

$$\boldsymbol{SRMSE_{max}}\ (mg.kg^{-1}) = \mathbf{0.027}\sqrt{\sum(\boldsymbol{E_m})^2}$$

The correction of the complete sheets (n=53) showed that the number of enrichments removed varied from 1 per sheet to a maximum of 4 per sheet and only 2 sheets (3.8%) reached this maximum, as indicated in the table 8. The validated spreadsheets represented 96.7% (n=174) and 33.3% of them (n=58) were validated after correction. The files that couldn't be corrected (n=6) were simply removed. The figure 8 describes the scheme of validation of the spreadsheets.

Table 8: Proportion of sheets corrected according to the number of enrichments removed (n=53)

Different level of correction	Classification			
Number of enrichments removed	1	2	3	4
Proportion of the corrected sheets	32.1 (n=17)	35.8 (n=19)	28.3 (n=15)	3.8 (n=2)

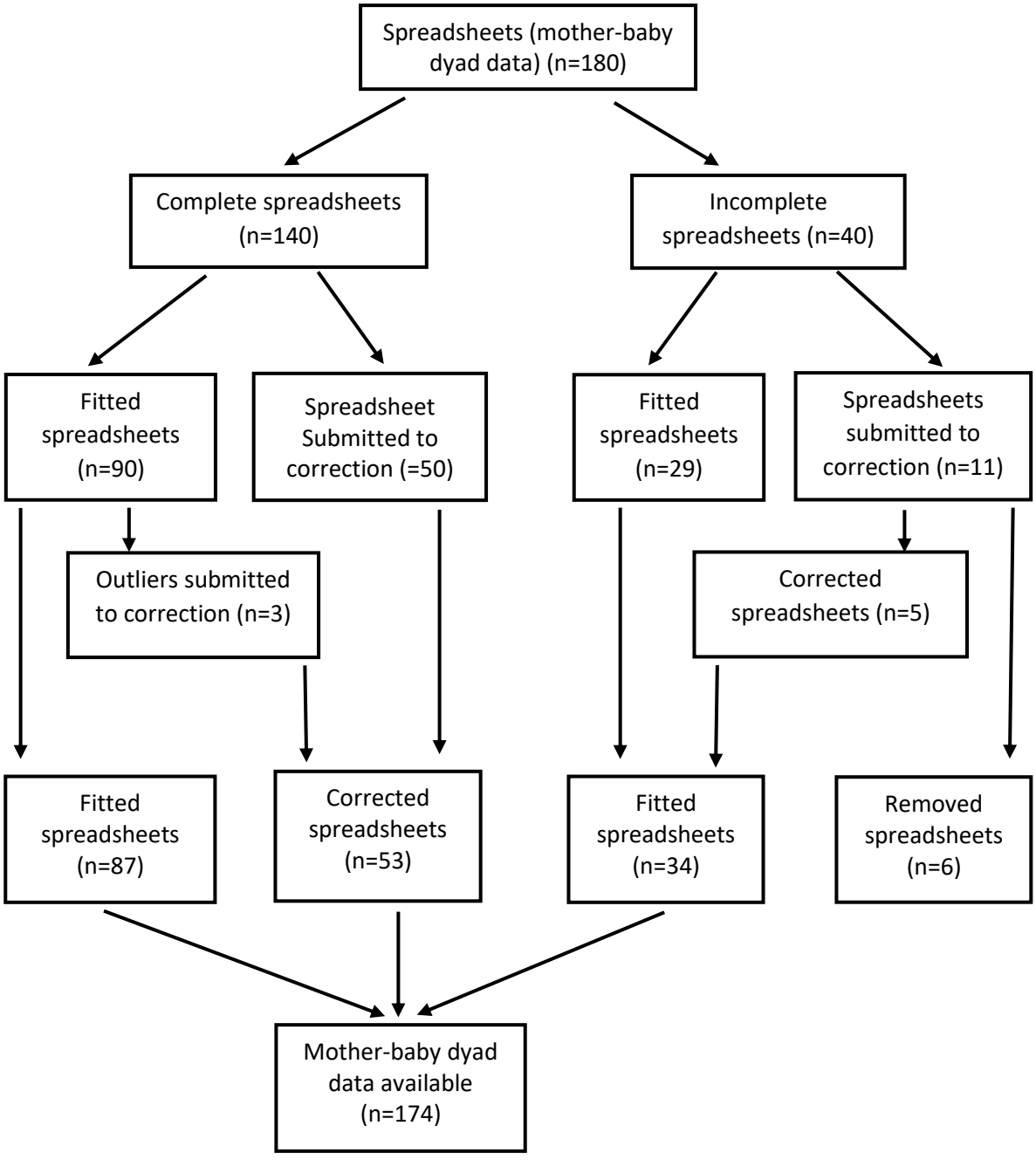


Figure 8: Data validation scheme

1.1. Effect of correction on the experimental SRMSE, HM and non-HM calculation

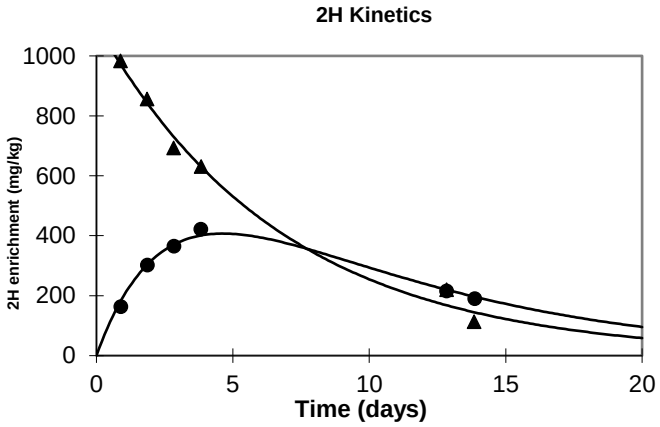
We founded the mean SRMSE (95% CI) decreased significantly ($p \leq 0.0001$, n= 53) from 49.78 mg/kg (46.35 mg/kg, 53.20 mg/kg) to 25.88 mg/kg (24.13 mg/kg, 27.64 mg/kg) after correction. We did not find any significant difference in the mean of HM as well as the non-HM after the enrichment correction. But when we considered the absolute difference at each mother-baby dyad level (decrease or increase in positive value), we observed that the mean difference of HM respectively non-HM that was 29.34 g/day (21.71 g/day, 36.97 g/day) respectively 24.13 g/day (17.4 g/day, 30.79 g/day) was strongly ($p \leq 0.0001$, n=53) different from zero. The table 9 shows the results before and after correction and the fig.10 gives an example of kinetic curve before and after correction.

Table 9: SRMSE, HM and non-HM before and after correction (n=53)

	Before	After	P	Absolute difference	P
SRMSE (mg/kg)	49.78[1] (46.35, 53.20)	25.88 (24.13, 27.64)	0.0000[a]	_	_
HM (g/day)	725.0 (656.75, 793.24)	727.4 (659.30, 795.64)	0.8047	29.34 (21.71, 36.97)	0.0000[b]
Non-HM (g/day)	79.15 (42.73, 115.57)	76.79 (40.68, 112.90)	0.7663	24.13 (17.47, 30.79)	0.0000

[1] mean (95%CI) all such value
[a] significant difference $p<0.0001$
[b] significant difference from zero, $p<0.0001$

a)



b)

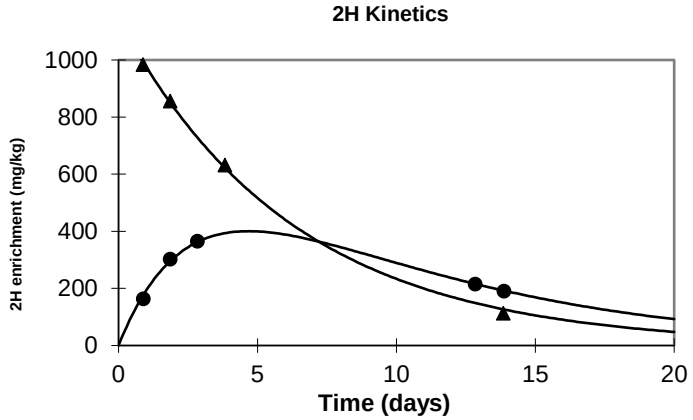


Figure 9: deuterium kinetic of the pair 34 before (a) and after (b) correction

2. Assessment of milk intake, exclusive breastfeeding practice and mother-baby pairs nutritional status

2.1. Participants' history

Fort-six mother-baby pairs were followed and 44 finished the 6 months measurement. They all have the same occupation which was rice production. The participant's social history showed that the mothers' mean age was 24.28 years, with 2 children alive for 3 pregnancies. Within the babies, 12.8% were born with low birth weight. The table 10 gives the participant's characteristic at inclusion.

Table 10: Participant socio-anthropometric characteristics at inclusion (n=46)

Characteristic	Mean	Median	Minimum	Maximum
Mother age (years)	24.28 (5.11)	25	17	40
Pregnancy (number)	3.35 (2.04)	3	1	9
Parity (number)	3.0 (1.86)	3	1	9
Children alive (number)	2.63 (1.30)	2	1	5
Baby birth weight (kg)	2.88	3	1.69	3.55
Birth weight<2.5kg (%)	12.8			
Baby birth length (cm)	48.76 (2.35)	49	39	52
Baby sex (M/F)	24/22			

2.2. Infants' nutritional status.

The babies' mean weight increased significantly (p=0.0000) from 2.98 ± 0.53 kg at birth month to 5.44 ± 0.81 kg at 2-3 months, then to 6.55 ± 0.93 kg at 4-5 months. All the babies doubled their birth weight at 4-5 mo. Malnutrition was present during all the follow-up according to all the indicator. The table 11 summarizes the evolution of the nutritional parameter up to 6 months.

Table 11: Infants' nutritional status up to 6 months

Nutritional Parameter	<1 month (n=46)	2-3 months (n=45)	4-5 months (n=45)	6 months (n=44)
Age	0.26 ± 0.17	2.33 ± 0.20	4.43 ± 0.26	6.20 ± 0.25
Weight (kg)	2.98 ± 0.53[a]	5.44 ± 0.81 [a,b]	6.55 ± 0.93[b]	6.85 ± 1.02
Length (cm)	48.93 ± 2.29	57.89 ± 2.45	62.55 ± 2.56	66.28 ± 2.59
WLZ	-0.65 ± 1.33	0,12 ± 1,17	-0,11 ± 1,16	-1,12 ± 1,20
WLZ< -2 (%)	18,6%	2,2%	8,9%	22,7%
WLZ<-3	2,3%	0,0%	0,0%	4,5%
LAZ	-1,04±1,30	-0,43±1,18	-0,65±1,13	-0,37±1,14
LAZ< -2 (%)	19,6%	10,9%	13,3%	4,5%
	6,5%	4,3%	6,7%	2,3%
WAZ	-1,08±1,19	-0,33±1,22	-0,57±1,17	-1,12±1,21
WAZ<-2 (%)	17,4%	8,7%	6,7%	20,5%
	6,5%	4,3%	4,4%	4,5%

*3 WHO flag data
Significant difference [a] p=0.0000 and [b] p=0.0000

2.3. Milk intake

The HM intake increase significantly (p= 0.0005) from 559 ± 208.2 g/day at birth months to 848.5± 175.6 g/day at 2-3 months and reached the maximum of 923.1 ± 184.2 g/day and (p=0.004) at 4-5 months when the non-HM was minimal (9.5 ± 67.4 g/day) as indicated in table 12.

Table 12: Fluid's intake up to 6 months

Fluids' intake	< 1 month (n=43)	2-3 months (n=43)	4-5 months (n=43)	6 months (n=42)
HM (g/day)	570.0 ± 208.2*, [a]	848.5 ± 175.6 [a, b]	923.1 ± 184.2 [b]	893.8 ± 198.1
Non-HM (g/day)	40.5 ± 56.0	59.2 ± 15.6	9.5 ± 67.4	187.5 ± 191.6
SRMSE (mg/kg)	27.1 ± 5.5	22.1 ± 6.2	21.63 ± 6.5	22.5 ± 6.8

*mean ±SD, all such value

Significant difference [a]p=0.0005, [b]p=0.004;

2.4. Exclusive breastfeeding up to 6 months

The EBF practice, as stated in table 13, was optimum at 4-5 months with 88.89% based on cross-sectional measurement. But when we analysed breastfeeding practice through longitudinal measurement, the proportion of infant who were exclusively breastfed from birth up to 4-5 months was 55% (95% CI=39.6; 70.4).

Table 13: Evolution of EBF rate from birth up to 6 months

Assessment*	<1 month (n=43)	2-3 month (n=43)	4-5 months (n=43)	6 months (n=42)
Cross- sectional	79.1 (63.7-89.0)[1]	73.9 (61.2-86.6)	88.8[a] (78.8-98.0)	35.7[b](21.2-50.2)
Longitudinal	-	60.5 (45.9-75.1)	55.0[a] (39.6-70.4)	13.9[b] (2.6-25.2)

*the EBF rate was determined using 86.6 $g.day^{-1}$ as non-BM intake cut-off [91]
[1]percentage with 95% CI in parentheses, all such value
Significant difference [a] p=0.0007 and [b] p=0.02

2.5. Maternal body composition

According to the BMI, the proportion of underweight was low during the follow-up with a range of 2.02 to 6.82% at 6 mo) and overweight varied from 13.04% (2-3 mo) to 18.18% at 6 mo; while the FMI indicated that fat deficit was very high, with a range of 59.09% to 65.91% and the over fat varied from 4.55% to 13.95%. The table 14 gives the anthropometric indicators of maternal nutrition and their body composition is consigned in table 15. The haemoglobin measurement as indicated in table 16 shows that global anaemia was persistent from birth (60.87%) to 6mo (58.70%).

Table 14: Maternal nutritional status determined by anthropometry up to 6 mo

Indicators	< 1 month (n=46)	2-3 months (n=46)	4-5 months (n=44)	6 months (n=44)
Weight (kg)	57.50 ± 7.3[a]	56.8 ± 7.4	57.1 ± 8.1	56.9±8.9
MUAC (cm)	25.9 ± 2.4	26.1± 2.3	26.6 ± 2.3	26.5 ±2.6
BMI	21.9 ± 2.2	21.7 ± 2.4	21.8 ± 2.7	21.7±1.3
BMI<18.5	2.2 (0.3-14.8)	2.2 (0.3-14.8)	2.2 (0.3(15.1)	6.8 (2.1-19.9)
BMI>25	15.2 (7.2-29.3)	13.0 (5.8-26.8)	17.6 (8.9-32.4)	18.2 (9.1-33.0)

[a] Mean ± SD, all such values; [b] percentage (95% CI), all such values

Table 15: Maternal body composition up to 6 months

Body composition	< 1 month (n=44)	2-3 months (n=46)	4-5 months (n=44)	6 months (n=43)
TBW (kg)	32.3 ±3.4	32.1 ± 4.0	31.5 ± 3.3	31.7 ± 3.8
FFM (kg)	44.1 ± 4.6	43.8 ± 5.4	43.1 ± 4.4	43.1 ± 4.9
FM (kg)	13.4 ± 5.4	12.9 ± 5.0	13.9 ± 6.0	13.7 ± 6.3
% FM	22.3 ± 7.6	22.4 ± 6.7	23.7 ±7.2	23.3 ±7.5
FMI ($kg.m^{-2}$)	5.1 ± 1.9	4.95 ± 1.9	5.3 ± 2.3	5.2 ±2.4
Fat deficit	59.09	60.9	65.9	60.5
FMI<5, % (95%CI)	(43.6; 73.0)	(45.7; 74.2)	(50.3; 78.7)	(44.7;74.3)
Severe fat deficit	20.5	19.6	18.2	23.3
FMI<3.5, % (95%CI)	(10.7; 35.5)	(10.2; 34.1)	(9.1; 33.0)	(12.7; 38.8)
Fat excess	4.5	6.5	15.9	13.9
FMI>9, % (95%CI)	(1.1; 17.2)	(2.0; 19.0)	(7.5; 30.5)	(6.2; 28.5)

Table 16: Haemoglobin level of the mother up to 6 months

	<1mo (n=46)	2-3 months (n=46)	4-5 months (n=45)	6 months (n=44)
Hemoglobin level (mg/dl)	10.8 ± 2.1	11.6 ± 1.2	12.3 ± 1.4	11.7 ± 1.3
Global Anemia (%)	60.9	58.7	46.7	61.4
Hb<12	(45.7-74.2)	(43.6-72.3)	(32.2-61.7)	(45.8-74.9)
Severe Anemia (%)	8.70	0	0	0
Hb<8	(3.2-21.7)			

3. Revisiting how exclusive is exclusive breastfeeding

3.1. Participants' characteristic at the final analysis

From the original dataset (n=46), after excluding subjects with lack of valid breastfeeding data, there were n=36 mother-baby pairs that fitted with our analysis. The table 17 gives the general characteristic of the mother-baby pairs involved in the analysis.

Table 17: Participants' general characteristics during the follow-up (n=36)

Characteristics	<1 month*	2-3 months	4-5 months	6 months
Mother Age (years)	24.72 ± 5.21 (17-40)	_	_	_
Pregnancies	3.39 ± 1.82 (1-9)	_	_	_
Deliveries	3.11 ± 1.83 (1-9)	_	_	_
Children alive	2.67 ± 1.24 (1-5)	_	_	_
Mother Weight (kg)	57.0 ± 7.4	56.1 ± 7.6	56.4 ± 8.3	56.3 ± 8.9
MUAC (cm)	25.8 ± 23	25.8 ± 2.2	26.5 ± 2.3	26.4 ± 2.7
Hb (g/dl)	11.0 ± 12.0	11.7 ± 1.2	12.3 ± 1.5	11.7 ± 1.3
BMI (kg/m^2)	21.8 ± 2.3	21.5 ± 2.3	21.6 ± 2.7	21.5 ± 2.8
Baby birth weight (kg)	2.9 ± 0.4 (1.69-3.55)	_	_	_
Birth weight <2.5kg (%)	13.9	_	_	_
Sex (M/F)	(18/18)	_	_	_
Baby age (months)	0.3 ± 0.2	2.3 ± 0.2	4.5 ± 0.3	6.2 ± 0.2
Baby weight (kg)	3.0 ± 0.5	5.5 ± 0.8	6.6 ± 1.0	6.8 ± 1.0
WLZ	-0.71 ± 1.3	0.13 ± 1.2	-0.17 ± 1.1	-1.2 ± 1.2
LAZ	-0.8 ± 1.1	0.4 ± 1.1	-0.5 ± 1.3	-0.4 ± 1.0
WAZ	-0.9 ± 1.0	0.3 ± 1.2	0.5 ± 1.3	-1.2 ± 1.2
HM (g/day)	583.8 ± 210.5	848.1 ± 193.4	919.3 ± 185.2	900.9 ± 193.0
Non-HM (g/day)	36.2 ± 49.0	68.2 ± 174.4	7.4 ± 68.1	190.2 ± 198.7

*Inclusion and first follow-up

3.2. Comparison between different methods of assessment of exclusive breastfeeding

There was a significant difference of EBF rate between the MR and the DMT at 0-1 month (22.2% (95% CI:5.9-38.9), p=0.0047) and at 2-3 months (27.9% (95% CI:10.4-45.2), p=0.0016) (table 18). Only at 4-5 months, the difference was not significant.

Table 18: Maternal Report Versus DMT

	MR (%)	DMT % (95%CI)	Difference % (95%CI)	Exact McNemar P
0-1 Month	100	77.8 (60.5-84.9)	22.2 (05.9-38.9)	0.0078*
2-3 Months	100	72.2 (54.7-84.8)	27.8 (10.4-45.2)	0.0020*
4-5 Months	100	89.0 (72.9-96, 0)	11.0 (01.9-24.2)	0.1250
Total: 0-5 months (n=108)	100	79.6 (70.8-86.3)	20.4 (0.12-0.29)	0.0000

Using the DMT (table 19), there was a significant difference of EBF when the assessment was cross-sectional compared to the longitudinal measurement since birth (36.2% (95% CI:17.6-54.9), p=0.0002 at 4-5 months).

Table 19: DMT current status assessment versus DMT longitudinal assessment since birth

	Cross-sectional % (95%CI)	Longitudinal % (95%CI)	Difference % (95%CI)	Exact McNemar P
Month (n=36)	77.8 (60.5-88.9)	NA		
2-3 months (n=36)	72.2 (54.7-84.8)	61.1 (43.7-76.1)	11.1 (1.9-24.2)	0.1250
4-5 months (n=36)	89.0 (72.9-96, 0)	52.8 (36.0-69.0)	36.2 (17.6-54.6)	0.0002*

*p<0.05

Finally, the WHO model showed a significant difference of 26.9 (95% CI: 17.6%, 36.1%) with longitudinal assessment, as shown in table 20.

Table 20: DMT accumulating age (WHO model) versus DMT longitudinal assessment

	Cross-sectional accumulated[1] % (95%CI)	Longitudinal[2] % (95%CI)	Difference % (95%CI)	Exact McNemar P
Month (n=36)	77.8 (60.5-88.9)	NA		
0-3 months, (n=72)	75.0 (63.5-83.8)	61.1 (43.7-76.1)	13 .9 (4.5-23.3)	0.0020
0-5 months, (n=108)	79.6 (70.8-86.3)	52.8 (43.2-62.1)	26.9 (17.6-36.1)	0.0000

[1]This is a simulation of the WHO model by cumulating the measurements of different age and the samples sizes (n=36, n=76 or n=108) are given in term of number of observations. There were the same infants (n=36) followed at different periods as infants from different group age followed approximately at the same time.

[2]The longitudinal model is drawn from the sample size n=36 at 0-1 month transformed to n=72 for 0-3 months and n=108 for 0-5 months by duplicating and triplicating the observations of the longitudinal assessment presented in table 19.

3.3. Relationship between breastmilk intake and feeding mode

The HM intake was independent of feeding mode from 0 to 5 months, but there was an enormous difference of non-HM between the babies who were exclusively breastfed and those who were not ($p<0.001$ from 0 to 3 months) (table 21 and 22).

Table 21: Fluids' intake (HM and non-HM) according to the mode of feeding at each age.

		HM		Non-HM	
EBF group	Frequency	Mean (SD)	P	Mean (SD)	P
0-1 month					
0	8	571.13 (228.73)	0.8501	100.75 (14.20)	0.0000*
1	28	587.43 (209.37)		21.57 (40.26)	
Total	36	583.81 (210.54)		39.17 (49.05)	
2-3 months					
0	10	760.0 (251.76)	0.1064	271.60 (204.61)	0.0000*
1	26	880.46 (160.03)		-10.12 (67.80)	
Total	36	848.11 (193.36)		68.14 (174.43)	
4-5 months					
0	4	887.25 (180.23)		162.5 (17.92)	
1	32	923.31 (188.23)	0.7192	-12.00 (41.46)	0.0000*
Total	36	919.31 (185.20)		7.39 (68.14)	
6 months					
0	23	840.21 (193.69)		274.87 (203.33)	
1	13	755.00 (301.66)	0.0100*	40.31 (36.17)	0.0002*
Total	36	900.89 (193.04)		190.17 (198.72)	

Table 22: Fluids' intake (HM and non-HM) according to the mode of feeding since birth

		HM		Non-HM	
EBF group	Frequency	Mean (SD)	P	Mean (SD)	P
0-1 month					
0	8	571.13 (228.73)	0.8501	100.75 (14.20)	0.0000*
1	28	587.43 (209.37)		21.57 (40.26)	
Total	36	583.81 (210.54)		39.17 (49.05)	
2-3 months,					
0	4	836.50 (311.04)	0.9772	315.50 (303.87)	0.0003*
1	10	840.40 (255.89)		133.20 (180.40)	
2	22	853.73 (142.62)		-6.41 (63.85)	
Total	36	843.11 (193.36)		68.14 (174.43)	
4-5 months,					
0	0	_			
1	5	960.00 (192.46)	0.6420	39.20 (92.36)	0.2755
2	12	946.42 (178.97)		20.52 (86.71)	
3	19	891.47 (192.66)		-9.11 (43.13)	
Total	36	919.31 (185.20)		7.39 (68.14)	
6 months					
0	0	_			
1	3	755.00 (301.66)	0.0493*	211.33 (105.71)	0.2071
2	8	1009.13 (126.72)		297.50 (354.09)	
3	20	849.85 (174.28)		176.40 (125.48)	
4	5	1019.40 (195.47)		60.80 (22.54)	
Total	36	900.89 (193.04)		190.17 (198.72)	

The EBF group 0, 1, 2, 3 and 4 represent the number of times the babies were assessed to be exclusively breastfed through the different measurement since birth up to the indicated age. 0 represent the group of infants that were never EBF since birth, 1 the group of infants assessed to be EBF once since birth. So, the highest number group at each age is the group of exclusively breastfed infants since birth, as determined by the DMT. The EBF since birth are in the group 3 at 4-5 months and in the group 4 at 6 months

CHAPTER 5: DISCUSSION

1. Validation of DMT data

To monitor and improve infant and children's nutrition, evidences need to be raised. In fact, in the monitoring of breastfeeding evaluation for better intervention, calculating the quantity of HM intake in different setting and at different ages of the babies is not sufficient. It is also important to estimate the non-HM intake in order to have good classifications in term of EBF or non-EBF.

The deuterium dilution method is suitable to evaluate breastfeeding practice, and this technique is now used in many field studies [20, 26, 32, 86, 107, 109, 111, 112]. However, complete and accurate complex information is necessary to compute good estimates of HM and non-HM intake. Several samples are to be collected at different time points after deuterium intake. The more we will have time point data, the more accurate the model will be. However, the collected data is subject to metrological errors. Finally, it is better removing a piece of information which looks inconsistent than running probably wrong estimations. The square root MSE is still used as a principal tool to identify uncommon data. This lies as an indicator of the validity of the model and we even recommend mentioning it in publications.

This is of high of importance for breastfeeding evaluation as for better intervention not only we need to know with more precision the quantity of HM intake in different settings and at different ages of the babies but we also need more precision on the non-HM for better classification in term of EBF or non-EBF.

It is not practically possible to eliminate all metrological errors as several complex steps of the data production contribute, including field data production (anthropometry, saliva sample collection, dose administration) and laboratory analysis (standard preparation, cell filling). In our approach, we figured out a strategy which will make us get a maximum boundary of that error estimated by the SRMSE.

The principal limitation of this work is the determination of our reference data. In fact, we checked all the different spreadsheets we had and selected the most accurate ones, according to the fitting as a reference. This is quite a subjective approach. However, the main objective is to discuss how qualitative decision can be taken using the SRMSE. We proposed a comprehensive approach to determine a higher bound for this measure in order to check the quality of the data. One could apply

the method using a set of his own data as we did or previous datasets, which are validated and even published, can be used.

In the present work, we found out that, when the data is fitted with the model, the SRMSE is less than $0.027\sqrt{\sum(E_m)^2}$. This cut off will help to check if data fit to the model and to eliminate the unfitted enrichment data.

We did not provide a unique value as cut-off but a variable dependent on the measured enrichment, since the quality of the data is not only linked to the smallness of the SRMSE but also to the contribution of the error produced by each measured enrichment. In fact, the SRMSE could be calculated with some fitting enrichment with very low MSE combined with an unfitted enrichment with a high MSE that increases the SRMSE. That was the case with the 3 outliers in α value calculation. The SRMSE of one of them (32.89) was less than the $SRMSE_{max,}$ but it provided α value of 0.029 that was greater than the α_{max} due to only one enrichment with a greater MSE. In order to have a better result, the unfitting enrichment needs to be removed even if the SRMSE seems to be low. So, our method helps to track and eliminate the hidden unfitted data.

After correction by removing the enrichment line, the significant reduction of SRMSE showed that the correction is really important for the optimization of the results as it provides a best fitting. The example given in the supplementary file illustrates well the validation by correction procedure. Before correction, the SRMSE that was 74 mg/kg was higher than the cut-off (47 mg/kg). After correction by removing enrichment line, the SRMS was reduced to 24.9 mg/kg and was less than the new SRMS cut-off (42.2 mg/kg).

We did not directly find any significant difference between the mean before and the mean after correction of HM as well as the non-HM because of some individuals' values that may decrease when the others increase and their difference's mean became statistically null. The consideration of the absolute value of difference between data before and after correction of HM and non-HM at each dyad level showed the effectiveness of the correction on HM as well as non-HM). This is critical for establishing the status of breastfeeding in term of exclusive or non-exclusive. For instance, it is known that there is an apparent non-HM (25 ± 63 g.day^{-1}) among women declared to exclusive breastfeed [31] or else if the non-HM is less than 52 g.day^{-1}, women were declared to exclusive breastfeed [28]. Thus, the validation of the data is essential before applying any

classification as it helps to have a good estimation of the non-HM and avoid misreporting on exclusive breastfeeding practice.

In this work, we showed that, during breastfeeding evaluation studies, all the data calculated with the model for breast milk evaluation are not valid and should not be considered in the result. The simplest way to validate data from breastfeeding practice evaluation using the deuterium dilution technique is to calculate for each mother-baby dyad the value of $0.027\sqrt{\sum(E_m)^2}$ and to compare it with the experimental SRMSE. If the SRMSE (mg.kg^{-1}) is above $0.027\sqrt{\sum(E_m)^2}$, so it is necessary to correct it. The best way to correct the data is to reanalyze the samples in the laboratory if it is possible to do so and to enter the new enrichments. The second possibility is to remove at maximum 6 enrichments line (3 for the mother, 3 for the baby) according to the criteria that we described above and our result showed that it is possible to have good results by removing less than 6 enrichments as the maximum was 4 (2 for mother and 2 for the baby) and that concerned only 2 sheets. Then the SRMSE is refined using the solver function. After that, if the SRMSE remained high, so the spreadsheet should be excluded from the result.

With this method, we validated 96.7% of our final data within 48.3% (n=87) that were originally good. When we corrected the sheets, we made only minor modification by removing 1 or 2 enrichments in 67.9% (n=36) of the file and 3 enrichments in 28.3% (n=15). Only 3.8% reach the level of 4 enrichments removed. That is very minor comparing to the 6 enrichment that could be removed in each sheet.

So, our results also showed the excellence of the first data provided by the field activities (anthropometry, dose administration, saliva sampling) and from FTIR analysis.

We already mentioned that FTIR analysis has been done with the Shimadzu 8400S. With the latest generations of FTIR that are more sensitive and precise, if the field data collection is well conducted and the samples analysis well performed, the result should be better than those given by the Shimadzu 8400S. Then the results should be easily validated according to our methods.

The procedure is also applicable to the results generated by the IRMS but using only the spreadsheets specially conceived for IRMS data that are given in molar ratio not in weight ratio like FTIR data. [94]

2. Assessment of breastfeeding practice and babies' and mothers' nutritional status

2.1. Infants' nutritional status, breastmilk intake and exclusive breastfeeding practice

Knowledge of breastfeeding practice and infant nutritional status is of particular importance for developing interventional strategies to prevent under-nutrition during the first 2 years. Using the stable isotope technique for determining human milk intake, we assessed breastfeeding practice in a rural community of Burkina Faso.

Anthropometry showed that the babies' weight increased rapidly from first to 6 months, but malnutrition was present with the highest proportion of wasting at birth month (18,6%) and 6 months (22.7%). That could be explained by the fact some babies were born with relative low birth weight (all the babies with low birth weight were malnutrition at the first month follow-up) and some babies can also lose weight during the breastfeeding initiation and it take time to cover the double need. At 6 months, the period of introduction of complementary feeding, there could be some difficulty in introduction of other food while breastmilk intake decreased. According to UNICEF, wasting and other forms of acute malnutrition result from maternal malnutrition, low birth weight poor feeding and care practices, and infection exacerbated by food insecurity, limited access to safe drinking water, and poverty [113] and new evidence shows that stunting and wasting might already be present at birth, and that the incidence of both conditions peaks in the first 6 months of life[3].

We found out the breastmilk intake by the babies increased significantly from birth up to 4-5 months and it decreased after 6 months. The milk intake is similar to that found by other study in different countries. The mean HM intake was globally similar to that found from 10 countries in DaCosta study of 2010 [32] and others studies [32, 107, 111, 112] precisely at 3 months and 6 months in Morocco (741.9 ± 281.7g/day and 843.6 ± 415.6 g/day) [107] and at 6 months in Bostwana (838.09 g/day ± 248.09 g/day) [112]. Our evaluation has been done among mothers who agreed to exclusive breastfeed up to 6 months and contrary to their willingness and report, very early some mothers introduced other foods in the babies' diet as showed by the EBF rate that was 79.02% at first month and decreased to 55.0% after 4-5 months.

The decrease of exclusive breastfeeding until 6 months showed the real non-compliance of this practice by mothers who had accepted it. We find here the difficulty of implementing the EBF recommendation. It should be welcome in rural areas where living conditions are often precarious

(low income and lack of hygiene) as we know breast milk is free, readily available and reduces the risk of infection[114].

Probably, whatever their willingness, they are not enough along in their projects to exclusively breastfeed and they often find themselves helpless face to social and cultural stresses; so during this period, in order to prolong the EBF duration, they need more counseling and support over than that they received at health service [27]. While mothers have limited powers to decide, grandmothers and husbands have the key role in decisions about breastfeeding and shows the need to engage the support of partners and relatives through community-driven policies and integrated interventions that address social and cultural barriers throughout the prenatal and postnatal period [115].

Comparing the EBF rate of cross-sectional evaluation to that obtained during longitudinal assessment, we remarked a significant reduction ($p<0.001$) from 89% to 55.5%. Referring to the definition of EBF up to 6 months, the cross-sectional measurement overestimates enormously the real proportion of EBF. A very earlier study based on maternal report showed that a cross-sectional evaluation (single 24h recall) overestimates the EBF rate comparatively to recall since birth that is a longitudinal evaluation[104].

Our results suggest that a unique evaluation at a specific period is not sufficient to say that moms have really observed the practice of exclusive breastfeeding up to that period, even if the evaluation is done using deuterium dilution. This challenges us on the method applied to evaluate breastfeeding, especially regarding the WHO indicator of EBF. That needs more investigation in order to have the most robust method that could be applied for better evaluation of EBF.

2.2. Lactating mothers' nutritional status

Breastfeeding is advantageous because of the nutritional, immunologic, and psychological benefits for the infant and its health impact on the mother. By the same time, the nutritional status of the mother is important throughout this period. The mother's daily caloric intake must increase significantly in order to replenish the mother's nutrient and energy stores.

Breastfeeding from healthy, well-nourished mothers is the ideal food to meet the nutritional demands of full-term babies[116] promoting their growth and the development of their gastrointestinal, immunological and neurological systems [117]

In our study, the mothers were in majority fat deficient, including a big contrast between BMI and FMI. As BMI doesn't give any detail on body composition, it could often hide a real nutritional status. The fat deficiency of Kou Valley lactating woman could be explained by the fact they had intense physical activity they body acted as sportive women or like men as they lose energy every day in the crops and/or there had a poor diet in nutrient conducting to fat lost. So, they can't storage fat and it is the FFM that was very predominant in the body weight increasing BMI that hidden the fat deficit.

But the fact they are fat deficient is of high of importance. For example, the EFA cannot be synthesized by the human body from precursors and must be consumed in the diet, and in the case of babies, through breast milk [118]. EFA consumption in the maternal diet has a direct influence on their concentration in maternal plasma[119], facilitating the uptake and endogenous synthesis of EFA in the mammary glands [120] so the composition of EFA and other fat-soluble nutrients in breastmilk will depend on maternal intake[121, 122]. Unfortunately, we didn't evaluate the dietary intake.

Kou valley is a rural area with the particularity that people have always something to eat. They produce rice and other cereals continually all the year and they can sell or keep it as they want; they have their own business and by this way, their daily meal is always provided. So, the main problem that could be relevant in their nutritional status must be more the micronutrient deficiency than the lack of food.

The hemoglobin level is an important indicator of nutritional status and health. In exception of the 4th month, the hemoglobin was at mean lower than 12g/dl and the proportion of anemia was high during the entire follow-up. There are several causes of anaemia, including blood loss, iron deficiency and other micronutrient deficiencies (e.g. vitamin A, folate, vitamin B12 and riboflavin), inherited haemoglobin disorders (e.g. sickle-cell disease and thalassaemia), parasitic infections and other acute and chronic infections that cause inflammation [123]. Burkina Faso is malaria's endemic zone and the infection by the plasmodium always causes anaemia. That is why the women received treatment during pregnancy. Iron deficiency often occurs before anaemia and is considered being one of the most common forms of nutritional anaemia [124] In Burkina Faso, according to the national policy, women receive a supplement of iron and folic acid during pregnancy and this supplementation is expected to continue during breastfeeding. But it is difficult

to assess the real intake of supplements by mothers both during pregnancy and after baby birth. Furthermore, a simple iron supplementation only does not reduce anemia irrespective of the overall micronutrient status.

3. Revisiting how exclusive is EBF as determined by the DMT

The question on the assessment of exclusive breastfeeding has discussed earlier and recently [104-106]. The debate conducted by Ted Greiner [105] summarized well the accuracies, advantages, inconveniences and limitations of the different type of assessment of EBF based on maternal report. The DMT that could help to validate maternal reports on breastfeeding practice [28, 30, 31, 109, 125] is an objective method for breastmilk intake and EBF assessment [126] and it is recognized now as the gold standard for breastfeeding practice evaluation. Recently, the technique has been updated on how to assess breastfeeding with more precision by facilitating data collection, management and validation [91, 92].

Our study compared the results from several ways to assess EBF in surveys. We found that very early, in the 2 first weeks after birth, some mothers introduced in their babies' diet other sources of water than breast milk contrary to their report and that continued up to 6 months. The difference was 22.2% (95% CI=05.9-38.9) at 0-1 month, 27.9% (95% CI=10.4-45.2) at 2-3 months. So, using a 24-hour recall to measure current status may overestimate the proportion of exclusively breast-fed infants because some infants who are given other liquids irregularly may not have received them in the 24 hours before the survey [105] and contrary to the DMT, the maternal or caregiver report is subjected to social desirability bias. Unsurprising the EBF proportion of longitudinal measurement up to a specific period was significantly less than that got from the cross-sectional measurement performed at that period. The example of 4-5 months where EBF was 89% (95% CI=72.9-96.0) at 4-5 months' assessment and 52.8% (95% CI=36.0-59.0) at the assessment since birth up to 4-5 month, revealed well that situation. Our results showed that a unique evaluation at a specific age does not assess those moms have really practiced exclusive breastfeeding before that time or after that time even if the evaluation uses the deuterium dilution method. So, the cross-sectional measurement using the DMT also overestimates the real proportions of EBF. Finally, after drawing the model of WHO indicator, we clearly viewed that, according to the definition, only the longitudinal assessment gave the best evaluation of EBF. The WHO model could be applied using DMT as it includes infants of different age from birth up to 5 months. But since it

doesn’t consider what happened to each infant before and after the evaluation, there will be the same bias as when the evaluation is done using maternal report (single 24h recall), including then an overestimation.

Indeed, evaluation since birth could penalize, for example, people with cultural practice of pre-feeding during the first 3 days after birth [105] even if they feed normally their babies immediately after that period and up to 6 months but, that occurs if the assessment stands on maternal report. The DMT takes 2 weeks for the evaluation and the calculation of fluids intake is the average of 14 days feeding practice. In one way, it could overcome the difficulty link to first days pre-feeding practice among people with cultural practice that not really influence the baby's normal growth. In another way, it permits detecting the babies who are truly exclusively breastfed but were declared not to be so by their mothers [31]. And the cut-off of 86.6 mg/day of non-breastmilk water intake should cover the daily quantity of medicines in case here was medication given to the babies during sickness that could occur within the 2 weeks of evaluation.

In the study, we used the protocol of seven samples collection from each mother and each baby. The new protocol developed recently for EBF classification and still in investigation for milk intake assessment, involves only 3 samples collection that will be more field friendly [127]. The DMT is limited by the fact it can’t be applied continually as after each dose it is necessary to wait for at least 45 days before starting another dosing, but it is the most accurate method for breastfeeding evaluation since now and it has the advantage to give at the same time the maternal body composition.

The cost effectiveness hasn’t been discussed here, but it is possible to introduce the technique as a routine assessment during national nutrition survey as several countries received like Burkina Faso the technical support of the IAEA and they have local expert to conduct the study at the national level.

CHAPTER 6: CONCLUSION

In our work, we first showed that, during breastfeeding evaluation study, all the data calculated with the model for breast milk evaluation are not valid and should not be considered in the result. So, we gave a predictive SRMSE that could serve as cut off for data validation in order to help the end user of DMT to best evaluate breastfeeding. In the second work, we provided the first data on the quantity of milk consumed by the babies and we evaluated exclusive breastfeeding up to 6 months and determined the maternal body composition in a rural community in Burkina Faso. We remarked that there could be hidden inadequate EBF practice after comparing the cross-sectional data with longitudinal measurement. Finally, we found the more objective and accurate method that should be applied in exclusive breastfeeding assessment. Only the DMT applied in longitudinal assessment can assess objectively EBF practice.

So, we contributed:

- to improve the results provided by the dose-to-mother technique (DMT) by determining a simple method to validate breastfeeding;
- to provide new data on breastfeeding practice in Burkina Faso using a method different from that is routinely used during nutrition surveys in the country;
- to find the best method to assess objectively exclusive breastfeeding practice.

The study showed that the deuterium oxide dose-to mother technique gives a more realistic estimate of EBF practice according to its definition and the technique gives the best assessment when it's applied using a longitudinal method That is high of importance for better nutrition intervention among infant and young children. Our work reaffirmed the utility of the DMT and wanted to encourage the introduction of the DMT as a routine assessment of breastfeeding practice during the national survey.

ANNEXES: PUBLICATIONS AND PRESENTATIONS LINKED TO THE WORK

1. Publication

Article 1:

Validation of data provided by the deuterium oxide dose-to-mother technique for better evaluation of breastfeeding practice
OJFNR: May-2019: Page No: 08-16

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Validation of data provided by the deuterium oxide dose-to-mother technique for better evaluation of breastfeeding practice

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Abstract

We describe a simple method to validate data collected from a study using the deuterium oxide dose-to-the-mother technique for breastfeeding evaluation. We used human milk intake calculation spreadsheets (n=180). The calculation was performed by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby. We assumed that the validity of the results is as high as the square root mean square error (SRMSE) between calculated and fitted data is low. Based on the original spreadsheets that fitted well with the model (n=87), we developed a simple prediction of the SRMSE and we used it as cut-off to check, correct (by removing enrichment data) and validate or remove the other spreadsheets. We found a cut-off dependent on the measured enrichment (E_m) that was $\mathbf{0.027}\sqrt{\sum(\boldsymbol{E_m})^2}$. And the mean SRMSE (90%CI) of the fitted sheets was 23.37 mg.kg^{-1} (22.01 mg.kg^{-1}, 24.73 mg.kg^{-1}) with a maximum of 38.96 mg.kg^{-1}. After correction we noticed that the number of enrichments removed per file varied from 1 to 4. We observed within the corrected spreadsheets a significant reduction ($p \leq 0.0001$, n=53) of the SRMSE (90%CI) from 49.78 mg.kg^{-1} (46.35 mg.kg^{-1}, 53.20mg.kg^{-1}) before correction to 25.88 mg.kg^{-1} (24.13 mg.kg^{-1}, 27.64 mg.kg^{-1}) after correction. We also observed that after correction, the mean difference (90%CI) of HM respectively non-HM that was 29.34 mg.kg^{-1} (21.71 mg.kg^{-1}, 36.97 mg.kg^{-1}) respectively 24.13 mg.kg^{-1} (17.4 mg.kg^{-1}, 30.79 mg.kg^{-1}) was strongly ($p \leq 0.0001$, n=53) different from zero. Therefore, the correction is very important to optimizing the results.

Keywords: Breastfeeding; Deuterium; Excel spreadsheet; Square root mean square error; Validation.

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Article 2 [*submitted, under revision]*

Abstract

Knowledge of infant nutritional status and breastfeeding practice is important for developing a strategy that can help to improve infant nutrition. In this work we described exclusive breastfeeding (EBF) practice and the nutritional status of breastfed babies from birth up to six months in a rural community of Burkina Faso. Forty-six mother-baby pairs were recruited at the baby's birth and follow up to 6 months. Infants' nutritional status was determined by anthropometry and we used the deuterium oxide dose-to-mother technique to measure the human milk intake (HM) as well as the non-milk oral water intake (non-HM) by the babies. Malnutrition was present during all the follow-up, with a high rate of wasting at first month (16.3%), and 6 months (22.7%). The HM intake increase significantly ($p< 0.001$) from 570.0 ± 208.2 g/day at birth month to 848.5 ± 175.6 g/day at 2-3 months and reached the maximum of 923.1 ± 184.2 g/day ($p=0.004$) at 4-5 months when the non-HM was minimal (9.5 ± 67.4 g/day). Based on the cross-sectional measurement, the EBF rate of 79.1% at the first month was optimum at 4 months with 88.89%. But according to the longitudinal evaluation, this rate is reduced significantly ($p<0.001$) to 55.5% at 4 months. There was inadequate breastfeeding practice up to 6 months contrary to the engagement of the mothers. The study provided the first data on breastmilk intake in the country and showed that, according to the definition, the cross-sectional measurement globally seems to overestimate the EBF.

keywords: infant nutrition, milk intake, exclusive breastfeeding, deuterium oxide dose-to-mother technique

Article 3: *submitted*

Revisiting how exclusive is exclusive breastfeeding practice as determined by deuterium dilution method since birth up to 6 months

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Abstract

Exclusive breastfeeding (EBF) for the first 6 months is widely recommended by WHO and UNICEF as feeding practice in early infancy. This implies the choice of a robust method to ascertain EBF application by mothers. In this work, we compared different methods of assessment of EBF after using the deuterium oxide dose-to-mother technique in "Vallee du Kou", a rural community of Burkina Faso.

Forty-six mother-baby pairs were recruited and followed-up to 6 months. EBF assessments were performed through longitudinal assessment by the means of 4 cross-sectional measurements at 0-1, 2-3, 4-5, and 6-months using mother's report (24h recall) and the deuterium oxide dose-to-mother technique (DMT). We also used the DMT data to simulate the WHO model of assessment of EBF that cumulates different age groups and the different methods were compared using the McNemar Test.

The results showed that the maternal report overestimated the EBF rate for 20% ($p<0.001$) compared to DMT. When the DMT was considered, there was an overestimation using cross-sectional measurement (36.2%, $p<0.001$) compared to the longitudinal measurement. Based on the WHO model applied to the DMT, we showed the WHO model overestimated the EBF for 26,9% ($p<0.001$) as compared to the DMT longitudinal measurement.

Taken together, the study showed that the deuterium oxide dose-to mother technique gives a more realistic estimate of EBF practice of mothers according to its definition. And the technique gives the best assessment when it's applied using the longitudinal method. The DMT can therefore be used as a routine method for the assessment of EBF during the nutritional surveys or intervention programs.

Keywords: Exclusive breastfeeding, deuterium oxide dose-to-mother technique, rural Burkina Faso, McNemar test

2. Conferences presentation

Nadine Mireille Coulibaly, Jean-Bosco Ouédraogo.

Malnutrition and inadequate breastfeeding practice among mother-baby pairs during the first 6 months after birth in Burkina Faso. Poster DBMal 119.

International Symposium on Understanding the double burden of Malnutrition for Effective Intervention, IAEA HQ, Vienna- Austria 2018.

Nadine Mireille Coulibaly, Jean-Bosco Ouédraogo, Augustin Zéba and Serge Somda

Use of stable isotope techniques to assess breastfeeding practices in Burkina Faso: what has happened from birth up to one year of age?

Oral presentation O38, FANUS Conference, Arusha-Tanzania; May 2015

Coulibaly N, Somda SA, Zéba AN and Ouédraogo J-B

Contribution of stable isotopes to better understand breastfed infants' nutritional status in Burkina Faso: longitudinal study with body composition measurement at 12 months. IAEA-CN-217--141P

International Symposium on Understanding Moderate Malnutrition in Children for Effective Intervention, IAEA HQ: Vienna- Austria May 2014

Coulibaly N, Ouédraogo CT, Zéba AN and Ouédraogo J-B

Contribution of stable isotopes to validate the practice of breastfeeding in Burkina Faso

Poster 566, 20th International Congress of Nutrition, Granada Spain, 15-20 September 2013

Coulibaly N, Ouédraogo CT, Zéba AN et Ouédraogo J-B

Mesure de la quantité de lait consommé par les bébés et de la composition corporelle des mères allaitantes par la technique de dilution du deutérium

Poster 77, 16 -ème journées des Sciences de la Santé de Bobo, 7-11 mai 2012

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