MSc Program
Environmental Technology & International Affairs

THE COMPARISON OF NUCLEAR AND CONVENTIONAL TECHNIQUES TO TACKLE THE DOUBLE BURDEN OF MALNUTRITION

A Master's Thesis submitted for the degree of
"Master of Science"

supervised by
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09.10.2016, Vienna

Affidavit

I, **CECILIA WEISS**, hereby declare

1. that I am the sole author of the present Master’s Thesis, "The Comparison of Nuclear and Conventional Techniques to Tackle the Double Burden of Malnutrition", 55 pages, bound, and that I have not used any source or tool other than those referenced or any other illicit aid or tool, and
2. that I have not prior to this date submitted this Master’s Thesis as an examination paper in any form in Austria or abroad.

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Abstract

This thesis sheds light on malnutrition, especially regarding vitamin A status, breastfeeding women and infants and overweight. Different techniques are described which assess breastfeeding behaviour and vitamin A and total body energy expenditure. In those, conventional field (non-isotopic) methods are compared to nuclear methods. Additionally, the involvement of international organizations is discussed.

Diese Masterarbeit behandelt das Thema der Fehlernährung mit einem Fokus auf Vitamin A, stillenden Müttern und Kleinkindern und Übergewicht. Verschiedene Methoden um das Brustfütterverahlten, Vitamin A und Energieverbrauch zu messen werden beschrieben. Herkömmliche (ohne Verwendung von Isotopen) Techniken werden mit Nuklearen Methoden verglichen. Außerdem wird die Beteiligung, in dieser Thematik, verschiedener internationaler Organisationen erläutert.

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List of Abbreviations

CRP	coordinated research project
EWL	evaporative water loss
FAO	Food and Agriculture Organization
FFM	fat free mass
FM	fat mass
FTIR	fourier transform infrared spectrometry
GDP	gross domestic product
HPLC	high performance liquid chromatography
IAEA	doubly labelled water
IAEA	International Atomic Energy Agency
IBFAN	International Baby Food Action Network
ILCA	International Lactation Consultant Association
IRMS	isotope ratio mass spectrometry
LBW	low birth weight
MAM	moderate acute malnutrition
MDG	Millennium Development Goal
MUAC	mid upper arm circumference
NGO	Non-Governmental Organization
RBP	retinol binding protein
RBT	retinol binding protein

RE retinol equivalent	*"a unit used for quantifying the vitamin A value of sources of vitamin A, including both preformed retinoids in animal foods and precursor carotenoids in plant foods. RE is defined as 3.3 International Units of vitamin A."* (Medical Dictionary, 2015)
ROC	receiver operating characteristics
SAM	severe acute malnutrition
SDG	Millennium Sustainability Goal
SUN	scaling up nutrition
TBW	total body water
TC	Technical Cooperation
TEE	total energy expenditure
UN	United Nations
UNICEF	United Nations International Children’s Emergency Fund
VAD	vitamin A deficiency
WASH	water, sanitation and hygiene
WFP	World Food Programme
WHO	World Health Organization

1. Introduction

Today, about 805 million people worldwide are suffering from undernourishment. In other terms, this means one in every nine people is not consuming enough food in order to be healthy and to live an active life. This definition makes famine the number one health risk globally, ranked even before AIDS, malaria and tuberculosis combined. The upside of this dramatic fact is that hunger can be solved! There are enough food resources to feed the whole world's population. The problems that are preventing communities from reaching their share of food products are lack of tools, policies and allocation (WFP, 2015). On the other side of the spectrum, overconsumption of nutrients and food resulting in obesity also shows a tremendously increasing trend. According to scientific estimates, overnutrition will be the cause of two-thirds of global diseases by 2020 (Unite For Sight, 2015). For that reason this Master Thesis focuses on different techniques used to assess composition in order to obtain reliable data that enables the ability to implement health plans tackling malnutrition. Furthermore, the United Nations has decided to raise the eradication of extreme poverty and hunger to the number one Millennium/Sustainable Development Goal (MDG/SDG). Accordingly, this topic is very up to date and internationally important. These goals were agreed upon by the country leaders and the world's leading developmental institutions. Persistent malnourishment may lead to weakened maternal health and child mortality. Eliminating hunger also tackles these two other MDGs.

The double burden of malnutrition commonly refers to both over nourishment, i.e. obesity, and undernourishment, i.e. hunger. This shows that a condition of weakened development can have several causes, as is discussed later on. In the present Master Thesis, this term also includes the case of malnourished people who have no visible signs of malnutrition, but suffer from micronutrient deficiencies, also called silent or hidden hunger. Malnutrition is a condition of the human body where an inadequate amount of nutrients and energy is consumed. The human body is in need of approximately 45 nutrients, which must be supplied daily at the right quantities. A continuous deprivation of those nutrients may lead to health deficiencies (Atinmo and Uchendu, 2011). These 45 essential nutrients can be classified in six groups (Desai, 2000):

- Carbohydrates

- Vitamins
- Lipids
- Water
- Protein
- Minerals

The present study sheds light on the second group, specifically on Vitamin A.

1.1. Definitions

Whenever using the terms “malnourished” or “undernourished” in this thesis it is used in a general meaning of not meeting a healthy body composition. But malnutrition has a broad spectrum and different definitions. Each stage of malnourishment has its own indicators, these classifications have been created by the World Food Programme (WFP) in order to better distinguish each stage and be able to make better assessments of a population’s health status. The following groupings with the WFP definition can be found under the “Hunger Glossary”:

Table 1: Definition of Different Forms of Malnutrition

Hunger	The situation where one is not having enough to eat to meet energy requirements. Hunger can lead to malnutrition, however absence of hunger does not imply absence of malnutrition (WFP, 2015).
Malnutrition	Malnutrition results when a person’s diet does not provide adequate nutrients for growth and maintenance of the human body. Furthermore, malnutrition may occur when a person is not able to properly digest and utilize the consumed food due to an illness or malfunction. The spectrum of malnutrition is broad and includes undernutrition (too thin, too short, micronutrient deficiencies) and overnutrition (overweight and obesity), alongside all the stages in between these two extremes. “Unbalanced nutrition” would be a better term for overnutrition as it often appears together with micronutrient deficiencies (WFP, 2015).

Stunting	Worldwide, approximately one in four children under the age of five is stunted. An even higher number of school-aged children suffer from being stunted. Stunting has the origin in a gradual and cumulative process during the 1000 days window from conception through the first two years of the infant's life. This is the main difference between stunting and wasting. The latter can develop within a short period of time and is reversible. Due to a continuous poor diet or a permanently low intake of micronutrients and repeated infections, or a combination of the two, stunting may develop. As indicated, this severe nutritional status has permanent consequences. The result may be shortness of stature, poor physical health as well as immediate and long term morbidity and mortality. Low height-for-age is an indicator for stunting, which is measured by comparing the height of a child against the WHO international growth reference for a child of the same age (WFP, 2015).
Undernourishment	Is sign of an inadequate nutrient and energy intake. This is based on the FAO definition of hunger, namely to consume less than a minimum level of kilocalories. The WFP uses this definition and assesses this at population level by using national food balance sheets in order to determine supply of food or energy given to a population. Furthermore, they model how this available dietary energy is distributed across the nation. According to data from 2014, one in nine people globally are undernourished (WFP, 2015).
Underweight	Reflects stunting and wasting. Its indicator is a low weight-for-age measurement that is calculated based on comparing the weight-for-age of a child with the WHO international growth reference (WFP, 2015).

Wasting	Another commonly used synonym for wasting is “acute malnutrition”. This health status develops as a cause of recent rapid weight loss or a failure to gain weight. To measure wasting, the WHO international growth reference is used and the children’s weight-for-age and size-for-age are compared. The mid upper arm circumference (MUAC) is also a tool to determine whether children suffer from wasting. Wasting can be classified in two different degrees, moderate (MAM) or severe (SAM). Acute malnutrition indicates the severity of an emergency, due to its origin: illness, sudden or severe lack of food. Wasting is strongly related to mortality and therefore combated by many different organizations (WFP, 2015).
Micronutrient deficiency	A lack or low intake of vitamins or minerals, which are essential in small amounts for proper growth and metabolism. Hidden or silent hunger is a cause of micronutrient deficiency, where people consume enough calories, but do not have the necessary vitamins or minerals. This form of hunger does not always result visually, but it increases morbidity and mortality. Other negative impacts of micronutrient deficiency are infertility, blindness, lower cognitive development and decreased economic development. Silent hunger affects over two billion people worldwide (WFP, 2015).

Table 1, by author.

Looking at these definitions one can say that nutritional health is interrelated with the food intake, the quantity and quality of food and physical health.

Nuclear techniques: are part of our socio-economic development and an unavoidable part of our daily lives. Starting from the dried herbs we use to season our food (food security), to medical diagnostics and treatment (medical), luggage control at airports or industrial process control (security and control), nuclear and isotopic techniques are being used to facilitate and improve these processes. Many people are unaware of the environmental and health benefits provided by these applications, because the general public learns more about accidents in nuclear power than about the positive research and medical treatments (IAEA, 2004). Nuclear engineering comprises the science of nuclear applications, the processes and different technologies. Nuclear and isotopic technology has become a vital part of medical diagnosis and treatment (Berkeley University of California, 2016). This thesis will focus on the nuclear processes in comparison to conventional methods used in the nutrition and health care sector.

Isotopic and nuclear practices have led to a more sensitive way to monitor health and nutrition techniques. The most common nuclear techniques in nutrition research are (Miranda-da-Cruz et al., 2003):

- doubly-labeled water: predominantly utilized to estimate energy expenditure, vitamin A studies and protein turnover, which make it possible to efficiently calculate the conversion of food to the body's protein storage or growth.
- deuterium dilution: to mainly measure total body water, nutrient needs and nutritional status.

Stable isotopes used as labels are applied to monitor copper, calcium, selenium, magnesium, iron and zinc. The utilization of stable isotopes in the field of nutrition monitoring is essential. Taking into consideration that they are used correctly, their use bears no health risk. These techniques have been proven to be an effective and efficient method to help countries that are struggling with malnutrition. Through the output of nuclear methods, nutritional interventions adapted to the individual condition can be implemented and save lives. Particularly, African and South Asian countries participate in these tests in order to eliminate malnourishment (Miranda-da-Cruz et al., 2003).

Nuclear and isotopic applications rapidly increase in importance especially in nutritional and health science. This positive development is not only due to its cost-effectiveness but also because these techniques have the ability to reach a large number of people. In the field of micronutrient bioavailability, barely any other non-invasive method has the capacity to measure with such precision (Miranda-da-Cruz et al., 2003).

Throughout this master thesis the terms "nuclear" and "isotopic" are utilized as synonyms and are interchangeable.

Conventional Methods: in this thesis all methods not using nuclear nor isotopic techniques are summarized under the term “conventional methods”. This approach is being used to facilitate the understanding and have a clearer distinction between the selected methods when comparing them with each other.

1.2. Causes and Facts

Malnutrition can be caused by many different elements. Half a century ago, it was thought to be a problem only poor social classes were facing. Famine is still a burden faced only by the poorest communities in developing countries. However, now, developed nations also suffer from malnutrition, for instance nutrient deficiencies and obesity, just to name two. In disadvantaged societies poor diet and repeated infections are the major reasons of malnutrition. These causes are directly connected to the standard of living of the aforementioned people, their environmental conditions and whether they are able to satisfy their basic needs such as nutrition, health care and housing. Diseases have in these contexts two connections, on the one hand they are a precursor and on the other hand they are the result of malnutrition (Blössner and Onis, 2005).

Persistent malnutrition can cause morbidity and also mortality, even though it is the direct cause of death in a few cases. At the beginning of the twentieth century more than half of all child deaths in developing nations were due to child malnutrition. This corresponds to 10.8 million children in 2001, according to a study by the World Health Organization conducted in 2004. The nutritional status is influenced negatively by disease with an environmental component as has been proven by evidence. As for instance, those spread by insects, protozoan vectors or caused by an environmental deficiency in micronutrients. The environment also has an even more influential role in relation to these aspects. Adverse environmental conditions have tremendous effects on a human’s nutritional status, such as the pollution of the environment leading to the destruction of the ecosystem, loss of biodiversity, climate change and the results of globalization. These developments stand in correlation with the increasing numbers of health hazards and all affect the nutritional status. The environmental carrying capacity is at its limits and the ecological balance is at the brink of a breakdown. This results in impaired food production patterns, which then promote an unbalanced diet and the intake of non-nutritious food. In contrast to this, the environment itself can also be negatively influenced by a population's dietary deficiencies. It is evident that until inhabitants cannot meet their basic needs or nourish themselves properly, they are not able to conserve the environment. This leads to supplementary health problems and deprivation - a viscous cycle. Thus malnutrition can be responsible for poverty due to decreased human efficiency and coping abilities. This provokes a series of other problems such as the reduction of economic and social development. It favors the utilization of unsustainable resources and environmental degradation (Blössner and Onis, 2005).

A main focus when talking about malnutrition should be shown on some of the most vulnerable members of society: infants, children and women. Women are a crucial element as it is through them that the next generation is born and the effects of malnutrition are propagated. A well nourished mother is key to a healthy born child. Therefore, particularly in developing countries malnourished women give birth to low birth-weight (LBW) babies. These babies are more prone to suffer from disease and premature death. This promotes the impairment of the work force and with that economic development, and the cycle of poverty and malnutrition is propagated (Blössner and Onis, 2005).

1.3. How to Combat Malnutrition

Any action to combat malnutrition must address the cause directly or indirectly. An intervention such as dietary intake provides up to 40 nutrients and directly targets malnutrition. Illness prevention is an example of an indirect measure. Illnesses increase nutrient needs and pose a major problem in societies that already tend to suffer from silent hunger (de Pee et al., 2015).

More specifically, ways to better address wasting, stunting and hidden hunger are directly through nutrition-specific interventions or indirectly through nutrition-sensitive ones. Nutrition-sensitive actions tackle the basic underlying causes. To improve and prevent malnutrition, a multi-sector approach is required, which means both direct and indirect interventions must be taken. This strategy is also emphasized by the Scaling Up Nutrition (SUN) Movement. When immediate action must be taken in emergencies, this approach is particularly difficult (de Pee et al., 2015).

Nutrition-specific interventions are, for instance, supplementary feedings, support to infants, young children and pregnant women. Nutrition-sensitive actions such as improving food security and livelihoods, water, and sanitation and hygiene (WASH) can positively influence some origins of malnutrition, especially the ones caused by illnesses. The combination of both sensitive and specific methods can, as research shows, prevent acute malnutrition (de Pee et al., 2015).

1.4. Critical Window of Opportunity – 1000 Days Matter

The paradigm of the first 1000 days of a child's life is referred to as the "critical window of opportunity." It starts with conception and lasts until the child's second birthday, or 24 months post birth. This crucial time period in developmental growth and neurological advancement lays the foundation for the child's mental and physical well-being during its entire life. There is vital research, much of it covered later in this paper, correlating the significance of breast feeding with childhood nutritional growth and brain stimulation over the course of the 1000 day critical window.

"Infancy and toddler life, through the first 24 months after birth, is a unique period during which human milk is recommended as either the exclusive source of nutrition (6 months)... (Solomons and Vossenaar, 2013)."

Nutrition-specific and nutrition-sensitive strategies during the critical window of opportunity can reduce the risks of stunting, wasting and micronutrient deficiencies. This is also recognized as the first line of prevention against malnutrition (de Pee et al., 2015). Breastfeeding offers the opportunity and the ability to shape healthier and more prosperous futures in youth. The adequate nutrient and energy intake during pregnancy and the first two years of life can have a serious impact on an infant's ability to grow. It can also help to decrease poverty and increase stability and prosperity (thousanddays.org, 2015).

The New York Academy of Sciences authors Harlod Alderman et al. (2014), described in their article on, "*Economic perspectives on integrating early child stimulation with* *nutritional interventions*”, that the time period in early development, the 1000-day critical period, through nutrition and brain stimulation correlates with economic development and has substantial economic returns. If the population is healthier, education programs are more successful and the workforce is more effective. Economic returns have vast influences on societal growth and socioeconomic development through self productivity in adults. Similarly, the emotional development in infants and toddlers shapes their entire lives into adulthood. By examining different periods in child development, many correlations can be found between prenatal care and later adult productivity (Alderman et al., 2014).

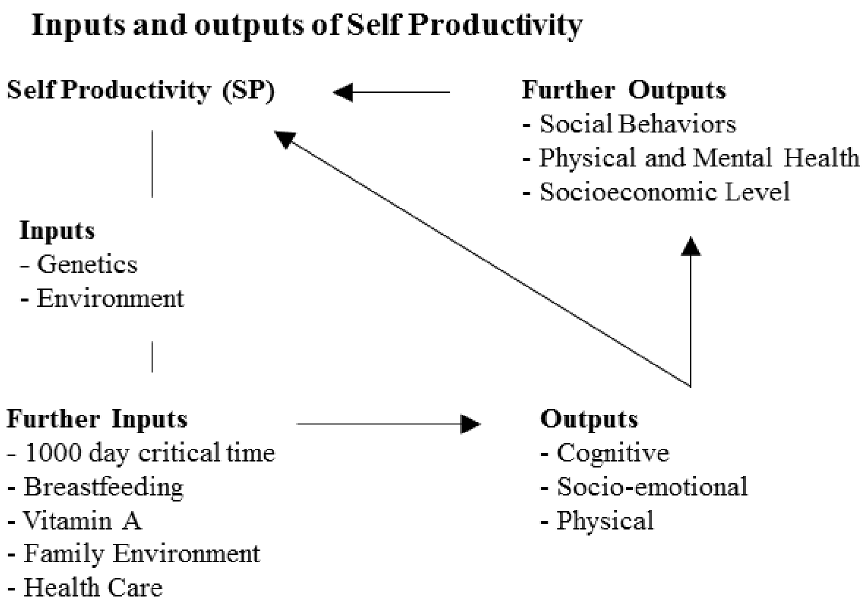

Figure 1, Inputs and outputs of self productivity, by author.

Various inputs in childhood development shape the outputs that can lead to health and productivity later on. Concrete inputs such as genetics and environment begin the cycle. Additional inputs such as 1000 day critical development, breastfeeding, vitamin A absorption, family environment and health care all play a role in shaping the cognitive, socioemotional and physical outputs. These inputs all effectively shape the self-productivity outputs. Self-productivity aligns with social behavior, mental and physical health and socioeconomic status later in adult life. Highly productive adults make up a successful and healthy economic environment. Getting the adequate secondary inputs such as 1000 day critical input and its components all play a direct role in a healthy adult life (Alderman et al., 2014).

The window of opportunity for ensuring a healthy adult life starts at conception, because during pregnancy undernutrition of the mother can have a tremendous impact on the development and physical and mental health on the unborn child. Undernourished fetuses have a higher probability of infant death and are more prone to suffer lifelong cognitive and physical deficits and/or chronic health problems. Being malnourished can be deadly for infants. It weakens a human's immune system and therefore lowers the barrier against common illnesses such as malaria, diarrhea and pneumonia in developing countries, which can be life-threatening. Investing in a better food intake in those 1000 days can positively influence a healthy and more productive future. This goes hand in hand with helping to break the cycle of poverty in the affected communities and regions (Alderman et al., 2014).

According to the organization 1,000 Days, evidence proves that the right nutrition during the window of opportunity can save more than one million lives per year, and also reduce the risk of developing diseases like diabetes and other chronic conditions that may occur later in life. Furthermore, it significantly diminishes the economic and human burden of illnesses such as malaria, tuberculosis and HIV/AIDS. Socially and economically adequate nutrition during that time improves the educational level and learning potential, thereby increasing a nation's GDP by at least two to three percent annually (Alderman et al., 2014).

These are the reasons why leading health experts, scientists and economists agree that an improvement in food intake during the critical 1000 day window is the best investment one can make to achieve a long-lasting positive achievement regarding global health. Solutions to this problem must be readily available, affordable and cost-effective. They must also, according to experts, include the accessibility of vitamins and minerals, especially to mothers and infants. The promotion of breastfeeding and other good nutritional practices are effective tools to combat child death due to malnutrition (Alderman et al., 2014).

1.5. Promotion of Breastfeeding

Within the last 20 years a decrease in child mortality can be observed. Nevertheless, still more than seven million children under five die annually from causes that are preventable. These are 13 percent of all deaths that could be eradicated by a simple and cost-effective action. Half of those deaths are newborns. Immediate breastfeeding right after birth would dramatically lower neonatal mortality rate. This is why many international organizations recommend to exclusively and immediately breastfeed. By doing so, mothers do not only help their infants to survive but also support healthy brain development, better cognitive performance and better educational achievements in the future. Breastfeeding additionally supplies the child with nutrients, which are important to protect the body from illnesses. Mothers that breastfeed their infants automatically give them a six times higher chance to survive their first months. For exclusively breastfed children the chances are even higher and their health status is better. They are 14 times less likely to die within the first six months than non-breastfed children.

Furthermore, the feeding of only mother’s milk can tremendously reduce deaths from diarrhea and acute respiratory infections which are, according to Lancet (2008), two of the main causes of infant death. Breast milk provides all the nutrients, vitamins and minerals that a child needs for growing. In the first six months, no other liquids or food are needed. Other elements are antibodies from the mother that help combat diseases. While drinking from the mother's breast a child also stimulates proper growth of the mouth and jaw, and produces secretion of hormones for digestion and satiation. Breastfeeding also has social benefits. It creates a special bond between mother and baby, which has positive repercussions for life, in terms of stimulation, behavior, speech, sense of well being, security and how the child relates to other people (UNICEF, 2015).

Organizations like WHO and UNICEF are strongly promoting breastfeeding due to the above mentioned benefits and also because in developing countries, the ones most affected, only 39 percent of infants under six months are exclusively breastfed. Those organizations recommend to start breastfeeding within the first hour after giving birth and to exclusively breastfeed for the first six months, and to continue breastfeeding for two years as a supplement to safe and adequate feeding (UNICEF, 2015).

Besides the benefits for the child, breastfeeding has also important benefits to the mother. If the breast is given to the child immediately after birth, it helps diminishing the risk of postpartum hemorrhage (severe bleeding after birth) and postnatal depression. In the long-run it also reduces type 2 diabetes as well as uterine, ovarian and breast cancer (UNICEF, 2015).

1.6. Hypothesis

There are many different approaches to tackle malnutrition. Especially regarding vitamin A status assessment, for instance, different international organizations recommend different techniques. This thesis aims to compare nuclear methods with conventional ones and to examine which one is the better option to be used to assess vitamin A, measure breastfeeding behavior and body energy expenditure.

1.7. Research question

The main research questions that guide this Master Thesis are the following:

- How effective, efficient and cost effective are nuclear methods compared to conventional methods?
- How widely and frequently are they used and is there a further potential to broaden their usage?
- How do different international organizations use these methods?

1.8. Methodology

The outlined research questions will be answered based on a review of scientific articles, text books, and literature published by organisations working in the relevant fields, with a strong representation of the IAEA. This is due to the role of the IAEA as a pioneer in the area of isotope applications for international development and the promotion of these techniques. The literature is used to provide the author with a foundation to perform a critical analysis and assessment.

The criteria for comparison are mentioned in the research questions above. They include the:

- effectiveness in terms of providing accurate and precise results, as well as reflecting natural conditions during the monitoring period, and
- efficiency in use, i.e. the simplicity of the procedure. Cost effectiveness is closely related to the resource versus result efficiency of the particular technique.

2. Monitoring of Breastfeeding Behavior

The World Health Organization (2014) defines “exclusive breastfeeding” as the habit of only feeding an infant breast milk throughout the first six months. This means no other foods, water or honey shall be fed to the baby, because additional feeding is the single, largest potential impact on child mortality, which can easily be prevented. This breastfeeding practice also incorporates the fact that the first “meal” of the newborn shall be taken within the first 30 minutes after giving birth. It can and should be continued for up to two years of age, i.e. the 1000 day critical window, and beyond. Exclusive breastfeeding is an essential pillar of a child’s survival and health. Breast milk is irreplaceable in that it provides the optimal nutrient, vitamin, mineral and energy intake to the child. This comes at the period, where the young organism is most vulnerable. Breast milk positively contributes to its growth and development, as well as immunization by delivering antibodies. Further, next to correct vitamin A uptake, breastfeeding directly contributes to a healthy adult life in physiological and social terms. Breastfeeding lays out the foundation for interpersonal bonds later on (WHO, 2014).

Even though it seems like breastfeeding is a simple and common way to feed a baby, much remains to be done to make exclusive breastfeeding the norm. According to WHO (2014), non-exclusive breastfeeding contributes to child mortality by 11.6 percent. Largely, children under five years of age are affected. These 11.6 percent amounted to 804,000 infant deaths in the year of 2011. Globally, only 38 percent of babies below six months old are exclusively breastfed (WHO, 2014). However, in the past, global rates of exclusive breastfeeding have been able to be raised. From 1985 to 1995, they increased by 2.4 percent per year on average, according to WHO. Afterwards, they subsequently decreased in most regions. Meanwhile, in 25 countries, rates increased by at least 20 percent, which WHO refers to as a global target (WHO, 2014).

In order to identify the need to promote breastfeeding among a population, several monitoring methods have been developed in the past. Conventional methods include test weighing and maternal expression, or recall. Due to a number of drawbacks related to limited precision and reliability of data and potential interference into natural patterns and practices, researchers have developed a method that uses stable isotopes to measure breast milk intake by the infant. This chapter describes the isotopic method, the conventional methods, a comparison of these, as well as the employment of the isotopic monitoring method and involvement of different international organizations in promoting this method.

2.1. Isotopic Method - Deuterium Oxide Dose-to-Mother Technique

The common isotopic technique applied to measure the intake of human milk is the deuterium oxide dose-to-mother technique. First performed by Coward et al. (1982), this is a precise and very informative method to assess whether an infant is breastfed exclusively, as in line with international guidelines, as well as how much milk the baby consumes. These data are essential to making an assessment of the nutrition and energy intake by the infant (IAEA, 2010). This simple technique uses deuterium (^{2}H) as a tracer to determine rates of water efflux from the mother and the baby as well as from mother to baby in order to monitor a baby's consumption of breast milk (Costa et al., 2010). According to the IAEA (2010), this method is increasingly being employed to monitor the progress of national breastfeeding promotion campaigns and infant nutrition.

^{2}H is a heavier stable isotope of hydrogen than the single proton that represents the most common form of the hydrogen atom. It is naturally present in our environment at low levels. Stable means that the isotope does not spontaneously undergo radioactive decay and does not emit any form of radioactive rays. As there is no radiation hazard, it is completely harmless at the amounts used for the purpose of monitoring breastfeeding practices (Slater, 2016).

According to the IAEA 2014 newsletter (Coulibaly and Munthali, 2014), the procedure goes as follows:

Table 2: Deuterium Oxide Procedure

Step 1	The lactating mother is weighed to 0.1kg, the baby to 0.01kg, following WHO instructions on how to measure an infant's weight.

Step 2	Baseline saliva samples are collected from both.
Step 3	The mother drinks the deuterium dose and feeds her infant as usual. The dose of tracer water consists of 30g of ^2H_2O (99.8 at % ^2H), regardless of the body weight of the mother.
Step 4	As the body metabolizes deuterium oxide (^2H_2O or D_2O) the same way as water, it is dispersed within the body water within a few hours. ^2H escapes the body in urine, saliva, sweat and human milk. Therefore, it flows from the mother's body to the infant via breastfeeding.
Step 5	Finally, the post-dose saliva samples are collected from both the mother and the baby. The monitoring period is 14 days, during which post-dose saliva (or urine) samples are taken on days 1, 2, 3, 4, 13 and 14 (IAEA, 2014).

Table 2, by author.

Isotope-ratio mass spectrometry (IRMS) or Fourier transform infrared spectrometry (FTIR) are used to measure the samples, i.e. the deuterium enrichment in the infant's and the mother's saliva. Due to its lower establishment and maintenance costs, particularly FTIR is an easy-to-use method and applicable in settings with limited financial and technical resources, according to the IAEA (2008). To interpret the enrichment levels determined in the saliva of the mother and the baby in the post-dose phase, a mathematical model is applied. The results reveal whether the infant was exclusively breastfed during the 14-day period, or not (Coulibaly and Munthali, 2014).

Figure 2 displays a steady state model with an unidirectional flow of water between two compartments, one being the total body water (TBW) of the mother and one of the child. As shown in the diagram below, the mother ejects water in form of human milk and other paths, such as saliva, sweat and urine. If exclusively breastfed, the baby receives water only from the mother in form of oxidation of human milk proteins, lipids and carbohydrates. If the baby is fed with water from other sources, the post-dose samples of the mother's and the baby's TBW and measured concentrations of ^2H_2O reveal this case (IAEA, 2010).

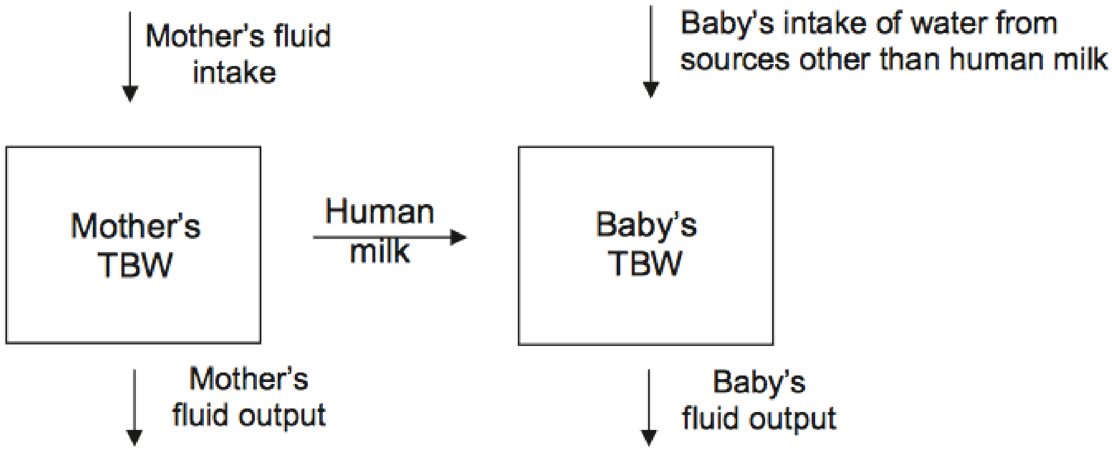

Figure 2: Two compartment steady state model of water flow in TBW of mother and baby. Source: IAEA, 2010.

The intake of milk and water from other sources than breastfeeding are calculated by feeding the measured deuterium enrichments into a model for water turnover in mother and baby. The measurements must be corrected for isotopic fractionation, as deuterium is lost via breath and transdermal evaporation more slowly than hydrogen, which is included in the calculation of total water output from the baby. This includes water exerted in form of sweat, urine, feces and breath, as well as atmospheric water absorbed by the baby's skin and lungs. Further, when evaluating the sampling data, the "Solver" function also corrects for the change in the baby's TBW during the two-week sampling period in which the little body grows. The child is weighed before the procedure and on day 14, i.e. at the end of the monitoring period (IAEA, 2010).

The graph below (Figure 3) visualizes how the deuterium enrichment declines in the mother's body water (circles) and appears in that of the child (squares) from where it rises within the first five days and then declines in the body water of both. This is because as the enrichment in the mother's milk falls, the enrichment in the infant's body declines as well. (Coulibaly and Munthali, 2014) The indicated points along the graphs show the points at which the post-dose samples are taken (IAEA, 2010).

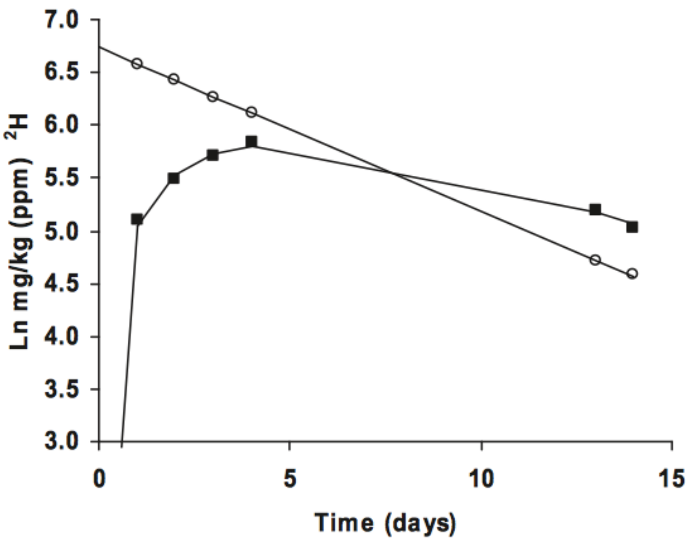

Figure 3: Natural logarithm (ln) of deuterium enrichment in the TBW of mother and infant plotted against the time from when the dose of $^{2}H_2O$ was consumed by the mother. Source: IAEA, 2010.

Figure 3 (above) displays a simplified view. According to the mathematical analysis, the curve of the mother's TBW enrichment with ^{2}H should follow an exponential decay (Bluck, Papanikolaou and Singh, 2011). As shown in the above graph, the child's curve starts from zero, reaches a maximum and then falls. In the last segment, the enrichment curve of the baby intersects that of the mother if it is exclusively breastfed. The difference between mother-baby pairs where the infant is exclusively breastfed and the case where the baby receives nutrition from other sources is illustrated in Figure 4 (below).

The two graphs in Figure 4 (below) display the difference in measured deuterium enrichment between a baby that is exclusively breastfed (Baby A) and a baby that receives milk or other nourishment from other sources, additionally to human milk (Baby B) (IAEA, 2010).

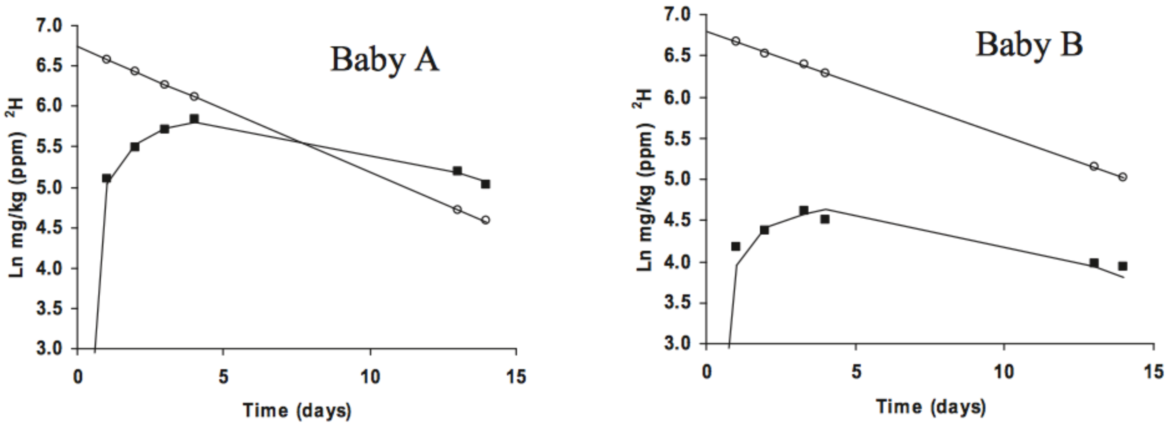

Figure 4: ^{2}H enrichment as registered in saliva of mother (circles) and baby (squares) for the case of an exclusively breastfed (left) and non-exclusively breastfed (right) child. Source: IAEA, 2010.

2.2. Conventional Methods

The most common conventional methods include test weighing and maternal expression, i.e. surveys through questionnaires and interviews. Beyond these, unannounced observations of interaction between mother and child, as well as monitoring the duration of lactational amenorrhea, are applied to validate reports of breastfeeding practices in communities. However, neither is considered a gold standard validation method (Moore et al., 2007). This chapter describes the two most common methods besides using isotopes.

2.2.1. Test Weighing

This broadly established method is based on the assumption that an increase in the baby's weight after feeding reflects the amount of milk consumed by it, as confirmed by a number of studies that validate the "*highly significant correlations between weight change and milk intake*". It is considered the easiest method to determine milk intake by breastfed babies. The infant is weighed before and after each feeding. Variations include combinations of maternal and infant test weighing to analyze nutrition from breastfeeding, or exclusive breastfeeding, based on combined weight differences. This method dominates monitoring milk intake rather than monitoring exclusive breastfeeding practices (Savenije and Brand, 2006).

2.2.2. Maternal Expression

The most common conventional method employed to monitor the extent and duration of breastfeeding practices as well as to evaluate the progress of breastfeeding promotion is via large scale surveys based on maternal expression. Here, mothers are given a question-and-answer form, or personally interviewed, and asked to respond whether their infant is exclusively breastfed, or whether it receives complimentary nourishment from other sources and if so, how much (Li, Scanlon and Serdula, 2005; USAID, 2011). Therefore, this method is purely based on maternal recall and their understanding of definitions of breastfeeding practices and scores on the form. The results are highly dependent on the conveyance of clear definitions of the apportionment of breast milk within the composition of total food intake of the baby.

2.3. Comparison

2.3.1. The Isotopic Method versus Test Weighing

The common conventional methods, namely test weighing and maternal expression, require little technical equipment and know-how. Establishment and maintenance costs are kept very low, which enables a broad application to communities with limited financial resources. However, it has a list of drawbacks that have given rise to the development of the method using deuterium.

Firstly, test weighing is time consuming as it requires to weigh either the infant, or both mother and infant, before and after each feed in order to measure the intake of breast milk. This time consuming practice interferes with natural feeding patterns and routines. Thus it runs high risk of distorting the test results (IAEA, 2010; Slater, 2016; Costa et al., 2010). Particularly, when babies demand feeding at night, practical challenges are posed when measurements must be taken before and after nursing at that time. Similarly, as infants are fed frequently during the day, the process of test weighing becomes very inefficient compared to the deuterium oxide dose-to-mother technique (IAEA, 2010), where measurement involves only a few steps from weighing mother and child twice each, offering the sample, as well as taking post-dose samples from the saliva or urine of mother and child only six times each. There is much less risk of altering habitual feeding patterns during lactation, which attributes much higher data reliability to the isotopic method.

Costa et al. (2010) also found that test weighing is "*difficult to apply in field conditions and unsuitable for large group studies*", precisely because of the highly time-consuming measurement twice every time the child is fed. Development workers, medical or other staff cannot take measurements several times a day for a large group of mother-baby pairs. If mothers themselves weigh their infants, researchers cannot be sure that the measurements are correct and comprehensive. Moore et al. (2007) found that overall the isotopic method provides much less of a burden on the young mothers. They also found that this technique does not require the research team to be directly supervised as the 12 saliva or urine samples speak for themselves within the model calculation. This further supports natural feeding behavior (Moore et al., 2007).

In regards to a clinical setting, test weighing seems more easily applicable as the test subjects are located within an institution and are limited in number. Savenije and Brand (2006) conducted a study on the accuracy and precision of test weighing within clinical conditions. They confirmed a strong association of the weighing results and actual breast milk intake by the infants and came to the conclusion that, while a slight systematic error is produced by evaporative water loss (EWL), the inaccuracies are "*too small to be clinically relevant*" (Savenije and Brand, 2006). However, their test results also yielded that the test weighing procedure is significantly imprecise. Due to the fact that a positive and negative error margin of up to 30 milliliters are possible, test weighing is too imprecise to determine the small changes in weight that are necessary to make a useful assessment of milk intake of babies over time. Savenije and Brand ascribe this to an insufficient sensitivity of the scales that are commonly used to measure the weight of the infants. According to them, test weighing should not be used for the purpose of monitoring breast milk intake of babies even under clinical conditions (Savenije and Brand, 2006).

Considering the findings cited above, the isotopic method has been found to be more precise and applicable in field conditions, as well as more accurate in that it interferes much less in the normal feeding behavior of the test subjects.

2.3.2. *The Isotopic Method versus Maternal Recall*

A common challenge reported when using the monitoring method of maternal recall, or expression, is a misinterpretation of terms used in the questionnaire, or interview. In their study, Moore et al. (2007) recorded higher rates of exclusive breastfeeding reported within a national survey in Bangladesh than observed upon using the stable isotope technique. They found that many mothers considered the term "exclusive breastfeeding" to include feeding their infants with both breast milk and water. This shows that the results of monitoring via maternal recall are subject to different understandings of definitions and scores. According to Moore et al. (2007), there is no "gold standard" among conventional methods to validate reports of maternal expression, but they see potential in the stable isotope method to provide such a service as it can also estimate the intake of fluids other than breast milk.

Motswagole et al. (2015) used the ^2H_2O dose-to-mother technique to validate records of breastfeeding practices in Botswana based on maternal recall. They discovered that mothers overestimated rates of exclusive breastfeeding at every time point monitored. Contrary to the high rates initially reported by the mothers, the rates of exclusive breastfeeding were low in Botswana. According to the research team, the stable isotope technique is a reliable method to crosscheck reports by mothers (Motswagole et al., 2015).

According to Li et al. (2005), existing research suggests that maternal expression is a valid and reliable source concerning the initiation and duration of breastfeeding, especially if breastfeeding is sustained over a relatively short period, such as three years. When it comes to the age at which food and fluids besides breast milk are introduced to the child's diet, the validity and reliability of this monitoring method are considered less satisfactory (Li et al., 2005).

2.3.3. *Cross-Cutting Benefits and Cost Efficiency*

The deuterium oxide dose-to-mother technique bears simultaneous benefits beyond monitoring breastfeeding practices. An important benefit of the deuterium oxide dose-to-mother technique is that the same baseline samples can be used to determine TBW content. Based on this, the fat free mass (FFM) and fat mass (FM) of the mother can be estimated, which reveal significant information on the lactating mother's nutritional status. This has strong implications for the nutritional status of the breastfed baby (IAEA, 2008). According to the IAEA (2010), the mother's body composition, i.e. her FFM and FM are derived as follows: FFM is measured in terms of deuterium dilution and FM is the result of subtracting FFM from the body weight. FFM in adults is considered 73.2 percent water (IAEA, 2010).

The results from the dose-to-mother technique allow an evaluation of the association between the human milk intake by the baby and the body composition of the mother. Further, they provide a basis to develop nutrition programs for communities of lactating women, as well as to quantify the transfer of nutrients and toxic elements likewise from the mother to the breastfed infant (IAEA, 2010). Besides the estimation of TBW with deuterium and the dose-to-mother technique to monitor human milk intake in babies, Hills and Davidsson (2010) rank the technique of oxygen-18 (^{18}O) to estimate total energy expenditure (TEE) as the third most significant method in developing and monitoring nutrition interventions around the world.

Considering the criterion of cost effectiveness, the isotopic technique may prove the most efficient method due to the possibility to monitor a range of other nutritional values, besides yielding precise results. Its precision and accuracy make direct supervision, as previously mentioned, and additional validation redundant, saving costs of both.

2.4. Involvement of Inerantional Organizations

2.4.1. The IAEA

Due to its expertise in developing and applying isotopic techniques in international development, the strongest propagator of the deuterium oxide dose-to-mother technique is likely the IAEA. The IAEA has conducted large scale Technical Cooperation (TC) projects to help Member States master the use of this technique and monitor human milk intake by breastfed infants in order to assess infant nutrition via breastfeeding (Slater, 2016). This includes the establishment of several FTIR units in carefully selected locations in Member States, predominantly in Africa (IAEA, 2008). Besides TC, the IAEA has an ongoing Coordinated Research Project (CRP) for PhD students precisely using the described dose-to-mother technique to assess the extent of exclusive breastfeeding (Slater, 2016).

The following section describes a number of projects and their target locations in three continents to illustrate the extent of the TC projects performed by the IAEA. The education program conducted by the IAEA is subsequently described.

Africa

Under the auspices of an IAEA project, researchers are currently studying baby feeding practices in 12 African countries (Slater, 2016). The study, which started in 2009 and will end this year, examines the infant's growth in combination with feeding practices. Here, the researchers have found that exclusive breastfeeding rates reported in questionnaires vastly exceed the rates monitored with the dose-to-mother technique (Slater, 2016). This is consistent with the results determined by Motswagole et al. (2015).

Latin America and the Caribbean

In a similar effort as described above, a team of researchers has been studying infant feeding practices in nine Member States in Latin America and the Caribbean. Starting in 2012 and ending this year, the project is evaluating breastfeeding promotion programs in these countries. Again, reported breastfeeding practices are compared to habits measured quantitatively by applying the dose-to-mother technique. Results confirm the trend detected in the African equivalent (Slater, 2016).

Asia and the Pacific

In this region, the IAEA project is studying infant feeding habits in 12 countries of the region. The project was launched in 2014 and will apply the deuterium oxide technique again to validate reports based on maternal expression. In all three regions, the objective is to evaluate programs that are promoting exclusive breastfeeding and to improve conventional assessment tools (Slater, 2016).

Education

As previously mentioned, the IAEA is also training Doctoral students. These students come from developing Member States, namely Burkina Faso, India, Kenya, South Africa, Sri Lanka and Thailand. Their training in nuclear techniques in nutrition uses laboratory capacity set up by the TC program of the IAEA (Slater, 2016).

Further, the IAEA has published newsletters and reports, including a related issue within the IAEA Human Health Series. This series is a publication providing information to medical practitioners, development workers, researchers and others interested in radiation medicine and isotope techniques. The issue on "*Stable Isotope Technique to Assess Intake of Human Milk in Breastfed Infants*" (IAEA, 2010) describes the procedure, sampling equipment, correct handling of the necessary equipment and of the samples, the data sheet with which to process sampling data and FTIR instrumentation, all in great detail. The issue also provides a stepwise guide to the correct sample analysis as well as points to look out for in order to deliver useful monitoring data of breastfeeding practices using the deuterium oxide dose-to-mother technique (IAEA, 2010).

2.4.2. WHO and Partners in Breastfeeding Promotion

The WHO is the second biggest player in exclusive breastfeeding promotion and has established the World Alliance for Breastfeeding Action (WABA) together with UNICEF. Core partners include The International Baby Food Action Network (IBFAN), La Leche League International, International Lactation Consultant Association (ILCA), Wellstart International and The Academy of Breastfeeding Medicine (WABA, 2016). WABA action is based on the Innocenti Declaration (UNICEF, 2016). The WHO formulates internationally recognized objectives and operational targets, including the Global Strategy for Infant and Young Child Feeding. Further, it translates the strategic objectives into national strategy and action plans as well as concrete policy (WHO, 2016).

The WHO itself does not conduct programs to monitor exclusive breastfeeding and human milk intake by infants via stable isotope methods (WABA, 2016). However, researchers are increasingly taking up this method to validate reports based on conventional methods. Thereby, they are supporting precise and comprehensive data coverage and promoting the use of the dose-to-mother technique (e.g. Moore et al., 2007). The WHO also administers the *WHO Global Data Bank on Infant and Young Child Feeding* (WHO, 2016). The WHO does not collect data itself but receives them from ministries of health, national research institutions, non-governmental organizations, United Nations organizations and online databases (WHO, 2016).

According to Savenije and Brand (2006), WHO views test weighing as a useful technique to estimate milk intake in breastfed babies. Their research lies ten years in the past and expert views within WHO may have changed, but in general the organization relies on the judgment and practices of its contributors for the supply with robust, accurate and precise data.

3. Vitamin A Assessment

Vitamin A is a vital nutrient for human immunity, reproduction, growth and general health. Biomarkers to assess its status are diverse due to the multiple purposes. Vitamin A liver reserves are an essential indicator of a population's health status as the liver is the major storage organ and reserves stored here are therefore considered the gold standard. Nevertheless, the measurement of vitamin A liver reserves is not financially feasible for population evaluation. Vitamin A biomarker status can be divided into two groups:

1. biological, functional and histological indicators
2. biochemical indicators

In the past, occurrence of xerophthalmia, a condition where the eye fails to produce tears, was used to determine the vitamin A deficiency. Even before medical damage to the eye, patients did suffer from night blindness and longer vision restoration times (Tanumihardjo, 2012).

Vitamin A deficiency is a public health problem, especially in developing countries. The most affected are infants and pregnant women of low income populations. According to WHO (2016), an adequate vitamin A status is essential to reduce illnesses and death in high risk areas. This is one reason, out of many, why countries need to assess this topic and introduce governmental health plans. Such plans can help nations to improve their health status and over time also improve their economic situation. Furthermore, it is necessary to educate the concerned group of population about this topic and teach the importance of breastfeeding and the regular intake of vital nutrients.

International organizations such as the IAEA and WHO shed light on this obstacle and recognize the importance of nation-wide interventions. It is WHO´s goal to globally eliminate vitamin A deficiency and the tragic consequences coming from it, including blindness, disease and premature death. The WHO points out that short-term interventions such as proper infant feeding are a good starting point, but must be backed with a long term sustainable plan (WHO, 2016). In order to estimate a nation's health risk level, certain measuring techniques are necessary. With the provided output of those tests, no matter if conventional or nuclear, governments shall elaborate a health plan and offer "well-being weapons." According to WHO, such a well-being weapon is the combination of breastfeeding and vitamin A supplementation. Countries also need to work out an enduring solution such as the promotion of vitamin A rich diets and the enrichment of oils with vitamin A (WHO, 2016).

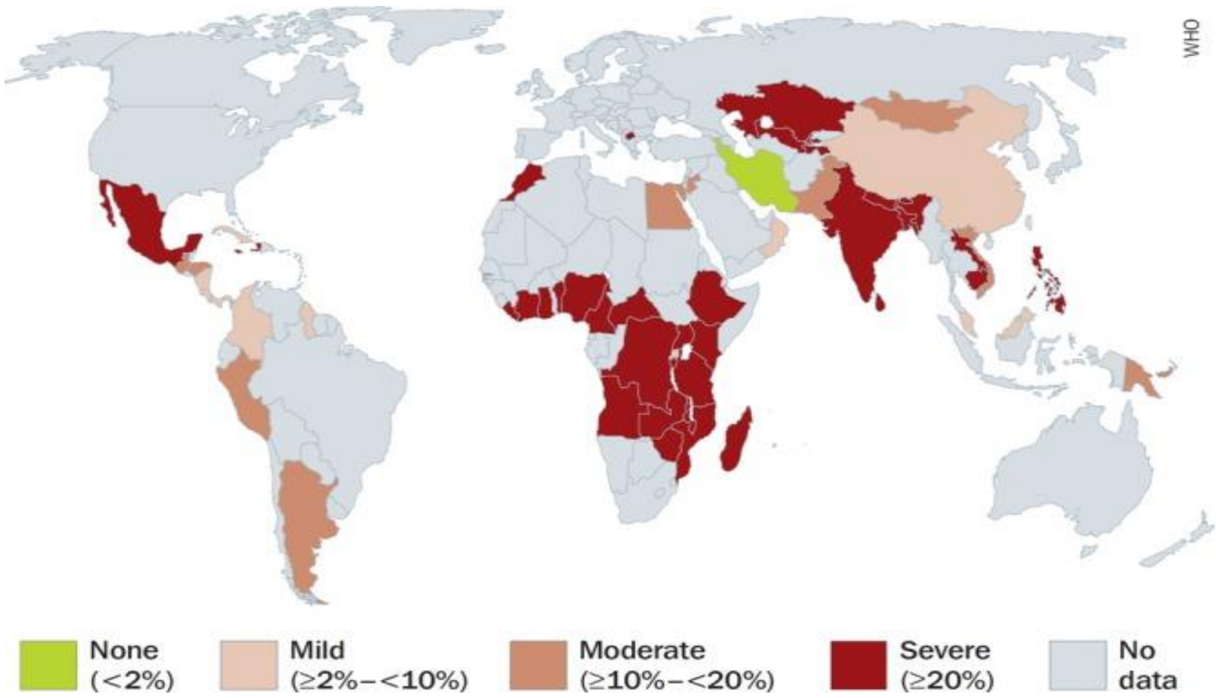

Figure 5, World map showing distribution and intensity of vitamin A deficiency. Source: WHO, 2016.

This Figure 5 shows the global extent of vitamin A deficiency as defined by the prevalence of serum retinol below 0.70 µmol/L. As shown in the legend of the above illustrated map, countries with two per cent below 0.70 µmol/L serum retinol do not count as suffering from vitamin A deficiency. Below two to ten percent they are considered to endure mild deficiency. A moderate shortcoming is defined as 10 to 20 percent below 0.70 µmol/L. If the measured serum retinol concentration is more than 20 percent below the reference number it is regarded as a severe deficiency (Kraemer and Gilbert, 2013). Furthermore, this world map shows the geographic location of concerned countries. Most nations suffering from severe shortage are developing countries. All grey colored countries have no data available regarding their national vitamin A level. This leads to the conclusion that the actual worldwide vitamin A deficiency extends to all continents. In order to retrieve such data, efficient and precise measurement methods are necessary. In the next two chapters a nuclear and a conventional method have been selected to show two possible ways for nations to evaluate their national health status.

The most commonly used technique today is the serum retinol concentration measurement, but it is homeostatically controlled until liver reserves become dangerously low. This means that as long as the lack of vitamin A is not yet critical, this method will not help identifying minor vitamin A deficiencies (Tanumihardjo, 2012).

This is why scientists have developed other methods in order to receive earlier vitamin A deficiency information already in stage of marginal shortage. One technique is the isotope dilution assay. This approach uses stable isotopes to trace total body reserves of vitamin A (Tanumihardjo, 2012).

Vitamin A assessment is conducted for all age groups, no matter of age or sex. As well as age and hormonal status are irrelevant for these methods.

3.1. Nuclear and Isotopic Techniques

Both radioactive and stable isotopes permit the determination of nutrient intake, body composition, energy expenditure, status of nutrients and the bioavailability of nutrients. These techniques are widely used in coordinated research projects (Venkatesh and Royal, 2002).

3.1.1. Isotope Dilution Technique

A common micronutrient deficiency is the Vitamin A deficiency (VAD). It is the cause a broad spectrum of illnesses and even deaths. VAD is particularly dangerous for pregnant and lactating women. Thus, this deficiency can be dangerous for both the mother and child. In order to prevent and improve this lack of nutrients, accurate techniques to assess vitamin A are needed in the field (IAEA, 2007).

There are diverse methods used and applied by different organizations and hospitals whereas the stable isotope dilution technique is the most commonly chosen one when it comes to assessing vitamin A. Through this method the nutrient can be evaluated in its efficiency and effectiveness. The data collected from these studies is then used to improve a society’s nutritional status. The stable isotope dilution method includes the process of administering a dose of stable isotope-labeled vitamin A to the test subject and after that determining the isotope ratio in plasma at specific times. In order to predict the total body vitamin A pool size, an equation by Professor James Olson and colleagues has been developed (IAEA, 2007).

Vitamin A is primarily stored in the liver. It is difficult to obtain these specimen from the liver and conventionally this is assessed through a biopsy, which is usually not justified since this entails a surgery. Indirect methods are serum retinol concentration response test and the stable isotope dilution technique. The latter one provides a quantitative estimate of the pool size of the exchangeable body pool of vitamin A. Through this information and a calculated liver-to-body weight ratio, assumptions of the vitamin A content in the body can be drawn. The stable isotope dilution method is the only indirect process that can provide quantitative data across the continuum of statuses, ranging from deficient to excessive vitamin A pool size. This means that this method can also be used in societies that suffer from an excessive vitamin A consumption, either through the intake of dietary supplements, vitamin A-rich or fortified foods. As already mentioned above, this method provides accurate quantitative data of the mean total body vitamin A body pool size for groups of subjects, but it is not intended to be used as a diagnostic method for individuals (Haskell and Ribaya-Mercado, 2005).

The choice which isotope will be used for the labeling depends on the target group, available instrumentation and cost. Deuterium labeled or ^{13}C-labeled vitamin A are both able to estimate the total body vitamin A pool size using isotope dilution technique. Deuterium labeled vitamin A compounds are commercially available, whereas ^{13}C-labeled vitamin A must be ordered as a custom synthesis from commercial suppliers of pharmaceuticals. The needed doses of Deuterium labeled vitamin A are generally larger than the doses of ^{13}C-labeled vitamin A. The reason for this is the difference in the sensitivities of the analytical instruments in the laboratories. Consequently, this means that Deuterium labeled vitamin A is more likely to perturb the endogenous vitamin A pool compared to the ^{13}C-labeled doses. Depending on the chosen paired stable isotope dilution technique, the minimum number of blood samples differs. If 13C is used, a supplementary blood sample is required in order to correct the residual 13C in the plasma from the first dose of labeled vitamin A.

Especially when working with infants and children it is desirable to choose a stable isotope labeled analog which requires less blood samples. Other variables that determine the chosen methods are the available instruments and costs (Haskell and Ribaya-Mercado, 2005).

The total body vitamin pool size is calculated with the following Olson equation:

Vitamin A pool size (mmol) = F * dose * (S * a * {(1 / D:H) – 1})

Table 3: Description of Olson formula

Variable	Description	Note
F	Factor for the efficiency of storage of orally administered dose	It is assumed to be 0.5
dose	Amount of labeled vitamin A in mmol retinol equivalent	
S	Corrects inequality between plasma and liver ratio of labeled to unlabeled retinol	Value of 0.65 is used, observed on mean plasma to liver ratio
D:H	Isotopic ratio of labeled to unlabeled retinol	In plasma
-1	Corrects for the contribution of mass of labeled dose to the total body vitamin pool	
a	Corrects irreversible loss of labeled vitamin A during mixing period	Adjusts for catabolism, calculated based on half-life of vitamin A turnover (140 days in adults)

Table 3, Source: Haskell and Ribaya-Mercado, 2005.

The orally administered dose of labeled vitamin A is not able to truly equilibrate with the endogenous vitamin A pool. Reasons for this are the catabolism of the dose during the 20-day mixing period and the constant intake on unlabeled vitamin A through dietary means. Recent consumed dietary vitamin A is preferentially secreted into the bloodstream, therefore diluting the labeled vitamin A in the plasma more than the labeled vitamin A in the liver (Haskell and Ribaya-Mercado, 2005).

Additionally, this technique can be used to detect quantitative changes in the total body vitamin A pool size in response to supplementary dietary interventions. The Olson equation displayed above gives a quantitative estimate in non-lactating and non-pregnant women. These two groups (one being non-pregnant and the other group comprising pregnant and lactating women) cannot be calculated with the same equation since their body is undergoing a different hormone-cycle and the results would deviate. No accurate equation has been found yet to quantitatively describe the total body vitamin A pool size for soon to be mothers and breastfeeding ones. Meanwhile, stable isotope technology can be used to gather qualitative data in these two target groups (non-pregnant versus pregnant and lactating). With three-day stable isotope dilution procedure qualitative data can be assessed for all groups (Haskell and Ribaya-Mercado, 2005).

3.1.2. Used Doses

The administered dose varies from the chosen isotope, the tested population and the instrument used, for instance through the detection limit of the mass spectrometer. Deuterium labeled retinyl acetate usually has the oral dose of five to ten milligram retinol equivalents (RE) for adults and 2.5 - 5 milligram RE for children. The ^{13}C labeled vitamin A is a rather new method and the oral doses for adults have been five milligram RE of 13C retinyl acetate. 13C has a relatively long half-life and can still be detected four years after dosing. Therefore, researchers are working to calculate smaller doses for future studies. If qualitative research is the aim of the study and blood samples are taken on day three, the doses can be cut by 50 percent. Another variable that influences the dosage is the development of instruments. The better the measuring devices get, the lower the administered doses (Haskell and Ribaya-Mercado, 2005).

3.1.3. Procedure

Once an isotope has been chosen, the analogs labeled with vitamin A are commercially available in powdered form. Vitamin A is light-sensitive. It is therefore important that the preparation of the next steps is performed in dim light. The labeled powder is accurately weighed, dissolved in ethanol and mixed with vegetable oil, in most cases corn oil. After evaporation of the ethanol the residue is filled into gel capsules, in this form it is easy for the target group to ingest (Haskell and Ribaya-Mercado, 2005).

Adults and children get those capsules directly, whereas infants and preschool children receive the dosage directly in their mouth using a positive displacement pipette with a disposable tip. Unrelated to the age of the target group a snack that is low in vitamin A, but high in fat is provided immediately after the dose intake. Through this, an enhancement of absorption can be guaranteed. Infants shall be breastfed right after administering the vitamin A labeled isotope. Non-breastfed infants must be provided with animal milk or formula (Haskell and Ribaya-Mercado, 2005).

The amount of sampled blood depends on the detection limit and sensitivity of the used instrument and the age of the target group. From adults and school-children, seven to ten milliliters are collected, and from infants three to five milliliters. The blood samples may clot prior to centrifugation, but must be protected from sunlight. Evacuated, foil wrapped blood collection tubes, which have been designed for the collection of plasma and serum, are used. In the cases where plasma is used, the tubes are inverted softly in order to enable a mixing of the blood with the anticoagulant. Afterwards, the foil is removed and the tube is centrifuged, to ensure a separation of serum and plasma the sample is spun 2800 * g for 15 minutes. The serum and plasma are both separated and transferred to two screw-cap vials, while the latter is flushed with argon or nitrogen gas. It is essential that the vials are covered in foil to protect the samples from light. After this centrifugation process, the vials must be labeled with a waterproof marker and stored at a temperature below -20 degrees Celsius. The samples have to be kept frozen during storage and shipment until they arrive at the laboratory for further examinations (Haskell and Ribaya-Mercado, 2005).

The above described blood samples are taken at 24 hours, three days and 20 days after being administered. In detail, this runs as follows:

24 hours - The first sample that is taken exactly a day after dosing gives qualitative information about the absorption process and mobilization of the labeled vitamin A from the liver. If at this point the plasma ratio of the labeled to unlabeled vitamin A is

low, this can be seen as an indicator of a reduced absorption and/or reduced mobilization of the labeled vitamin A to the liver. This knowledge is crucial when analyzing the data and interpreting the results (Haskell and Ribaya-Mercado, 2005).

Three days - This blood collection is important because after three days the plasma isotopic ratio of labeled to unlabeled retinol highly correlates with the estimates of vitamin A pool size and offers a qualitative estimate of the total body vitamin A pool. In population groups that are prone to infections it is recommended to use the three day sample as infections are less likely to occur within a three day sampling period than a 20 day one (Haskell and Ribaya-Mercado, 2005).

20 days - This sample gives quantitative results using the Olson equation (Haskell and Ribaya-Mercado, 2005).

3.1.4. How Widely Used

The stable isotope dilution technique has favorably been used in the United States, Bangladesh, China, Guatemala, Philippines, Peru and Nicaragua in order to estimate the body vitamin A pool size of children and adults and further to evaluate the effectiveness and efficiency of national vitamin A interventions (Haskell and Ribaya-Mercado, 2005).

3.2. Conventional Method

It is not possible to trace vitamin A body storage in urine. Also, blood levels are not a sensitive reflector and do not deliver intermediate status and body storage. The most commonly used conventional methods in the field are clinical signs and symptoms, for example night blindness and conjunctival dryness. One of the most precise vitamin A body storage measurement, regarding non-nuclear techniques, is the liver biopsy, which is mainly only conducted on corpses or while a patient has a different surgery (Journal of Nutrition, 1990). A by far less invasive conservative method is the serum retinol concentration technique, described in the following chapter.

3.2.1. Serum Retinol Concentration

A conventional method to calculate the vitamin A status of a human being can be the measurement of the serum retinol concentration. The assessment of this concentration requires high performance liquid chromatography (HPLC). This makes this application expensive, rarely available and technically demanding in developing countries. In areas with a lack of resources, specific serum retinol indicators are proposed as proxy markers of vitamin A. Such a chosen retinol indicator is the RBP – retinol binding protein concentration. RBP is a transporting protein which not only carries but also binds and delivers retinol to the target organs. It is produced in the liver and its secretion from there into the circulation is determined by the circulating retinol levels. Some researchers suggest that the ratio of the present RBP in the serum has a 1:1 molar ratio. This ratio is based on an experiment with rats and varies for humans. Studies have shown that the ratio is also dramatically influenced by inflammations, obesity, malnutrition, vitamin A status and impregnation. Hence the RBT:transthyretin ratio is not influenced by inflammations, for that reason it has been proposed to be the indicator of vitamin A status (Talsma et al., 2015).

The process of this method starts off by taking blood samples. A finger prick is also conducted to measure the hemoglobin concentration and the weight and height are taken of every individual in the target group. Six milliliter venous blood is obtained and immediately kept shielded from light and cooled down to two to eight degrees Celsius. At this temperature the blood samples are stored for about one hour and after that the centrifugation process starts, this takes ten minutes at 1200 g. After this step, the serum is stored for another four to eight hours at two to eight degrees Celsius and afterwards in liquid nitrogen until sending them off to the laboratory. For the duration of the transport the samples are kept at a temperature of -80 degrees Celsius (Talsma et al, 2015).

For the biochemical analysis the samples have been processed under dimmed yellow light. 200 µL sodium chloride and 400 µL ethanol (96% ethanol has been used) whereby the retinyl acetate has been used as the internal standard to 200 µL serum. In previous field studies every third sample was taken, if a sample was not sufficient the next one on the list was used instead, in order to ensure the same concentration range. Distribution of serum markers were examined visually by focusing on histograms. To determine the precision of proxy serum markers in detecting vitamin A deficiency, ROC (receiver operating characteristics)-curves were the technique utilized. To assess the diagnostic accuracy, optical differences in the area under the curve were analyzed (Talsma et al., 2015).

While conducting the statistical analysis, it was found that a combination of proxy markers have the better ability to distinguish between children with and without vitamin A deficiency. This was the result of many tests and by using the best proxy marker alone, after which other proxy markers were added. After the tests, the decision was taken to use a parsimonious model that only included markers with independent diagnostic value when used with others (Talsma et al, 2015).

After the results of 372 children were obtained, the following findings came to light:

- 18 percent of the zest participants suffer from vitamin A deficiency
- ten percent had inflammations (this number is lower than initially expected from the experts)
- the better test performances were received when different markers were used in combination and has the potential to be used widely (Talsma et al, 2015).
-

3.3. Involvement of International Organizations

3.3.1. The IAEA

The International Atomic Energy Agency has a division (Division of Human Health) that studies prevention, diagnosis and treatment of diseases through the use of nuclear methods. Nuclear medicine can be used in a broad area. One applied by the IAEA is in the field of nutrition. Here, they are working on stable isotope techniques to fight against malnutrition in all forms. Such nuclear methods are being used to assess body composition, bone mineral density, breastfeeding behaviour, total body energy expenditure, micronutrient bioavailability and, last but not least, vitamin A status (IAEA, 2014).

This international organization finances publications on stable isotope applications. Besides sponsoring research, the use of those methods in developing countries is supported. The stable isotope technique is one of the only methods that can assess the total body vitamin A stores without surgery. The IAEA helps with vitamin A studies and further supports a nutritional plan, for instance food fortification to increase vitamin A levels of a whole population. Additional studies are sponsored, for example the comparison of two different food preparations of the same product in two different countries. First, the micronutrient bioavailability is compared between the the countries and then the cooking habits. Through such research, public health interventions can be compared and also recommendations and predictions can be made. Additionally, food fortification plans can be justified more easily and the health benefits of a whole population can be demonstrated when compared to a region with similar nutritional habits that has already been given the fortified produce. The IAEA also strongly supports the use of stable isotopes to fight against child mortality. This is because this method is secure and less invasive, especially for breastfeeding mothers and infants. For this reason, the IAEA conducts many studies on a global level on children, in order to have more accurate data to make individualized interventions for the youngest (IAEA, 2014).

3.3.2. WHO

One of WHO's goals is to eradicate vitamin A deficiency and the consequences that come along with lack thereof, such as blindness and premature death just to name two out of many. The WHO recognizes the need of fast interventions with a quick result in combination with sustainable long-term interventions. Such rapid actions recommended include exclusive breastfeeding until the sixth month and vitamin A supplementation. These actions should be merged with a promotion of food that are rich in vitamin A and food fortification (WHO, 2016).

A high dose of vitamin A shall be supplied to deficient children. This is a cheap, simple and highly beneficial method to reduce child death by 23 percent. Furthermore, a reduction up to 50 percent for acute measles suffers. The WHO claims that it is important to start such interventions in the early stages of life. Studies show that the first six years are crucial and an adequate vitamin A level can reduce child mobility by 25 percent (WHO, 2016).

Food fortification is one of WHO's long-term projects, because it takes over where supplementation leaves off. An example for such an intervention is the fortification of the sugar cane in Guatemala. Through the use of the fortified sugar, families in need and high-risk groups have the opportunity to maintain their vitamin A body stores at an adequate level. This positive example pushed WHO to further implement similar projects. For example, in Africa and South-East Asia food fortification contributes to a better and lifelong health. (WHO, 2016).

4. Measuring Human Energy Expenditures and Metabolic Function to Combat Obesity

4.1. Why Measure Energy Expenditures

Obesity is “*characterized by excessive deposition of fat in adipose tissue*” (Rosado et al., 2013) due to a chronically elevated energy balance. This may come from excessive energy intake and/or changed body energy expenditure per kilogram of body weight, for example a lower metabolic rate. Obesity is considered a chronic disease with epidemiological concern and measuring energy expenditure provides a basis upon which to evaluate causal factors and to form customized nutritional interventions to prevent and treat the disease (Rosado et al., 2013). Understanding how energy intake and energy expenditure interact, as well as body composition and its energy stores, helps to develop strategies to reduce obesity, either cut out for individuals or characteristic groups of individuals.

According to Hill et al. (2012), success in reducing obesity requires that both energy intake and energy expenditure are modified, not only one of the two. They point out that if a human organism is tuned toward an energy balance at a high energy intake and expenditure (i.e. a high energy flux), food restriction as such will not reduce obesity. A high energy flux can be achieved by a high level of physical activity, which was historically often a permanent condition. Meanwhile, in today’s sedentary societies, Hill et al. (2012) see it increasingly achieved through excessive weight gain. In order to achieve an energy balance, the feasible goal is to prevent excessive weight gain, instead of focusing on weight loss. This is because physiology that functions at a high energy flux is highly resistant to weight loss. While only very drastic behavior changes can instigate and maintain weight reductions, the prevention of excessive weight gain is possible at small changes in behavior (Hill et al., 2012).

The study of energy intake and energy expenditure therefore supports the development of strategies based on the concept of energy balance to reduce obesity rates (Hill et al., 2012). In line with this, the measurement of energy expenditure enables the study of metabolic needs and dietary fuel utilization, as well as drug and emotional components to weight gain (Ja, 2005). Knowledge of these components helps to develop nutritional interventions with the highest possible efficiency and to make recommendations on nutritional and physical activity that are compatible with healthy lifestyles. This chapter reviews the most common conventional methods and the method using stable isotopes to assess energy expenditure in humans, followed by a comparison and an outline of the involvement of international organizations.

4.2. Stable Isotope Method

4.2.1. Doubly Labeled Water ($^{2}H_{2}{}^{18}O$/DLW)

According to DeLany (1997), this technique is based on the principle that after intake of a dose of DLW, the two isotopes mix into the total body weight (TBW) and then exit differentially from the body. Deuterium leaves the body as water, while ^{18}O leaves as water (H_2O) and carbon dioxide (CO_2). The production of carbon dioxide can be derived by subtracting hydrogen (H_2) elimination from ^{18}O elimination. The doubly labeled water (DLW) method is an accurate technique to validate other methods to determine the total energy expenditure (TEE) of "free-living" subjects, as opposed to subjects tested under clinical conditions (DeLany, 1997). The DLW technique was discovered for its current purpose by Lifson and McClintock in 1966. Since then it has gained momentum to where the IAEA refers to it as a "*gold standard*" among TEE measuring techniques, "*ideal for nutrition interventions in any field setting*" (IAEA, 2008). Similar to deuterium, ^{18}O is naturally present in water, in our food and in the body. Around 0.20 percent of all bound and unbound oxygen is ^{18}O (IAEA, 2008).

According to the IAEA (2008), the procedure is conducted as follows:

Table 4: Doubly Labeled Water Procedure

Step 1	The participant drinks a dose of doubly labeled water. This mixes with the components of body water within a few hours.
Step 2	Urine samples are taken over a monitoring period of 14 days and the isotope concentrations of H-2 and O-18 are analyzed.
Step 3	Calculation of energy expenditure from sampling data.

Table 4, by author.

As the body produces energy, hydrogen and oxygen are set free from the body, whereas oxygen is bound in both H_2O and CO_2 causing it to be lost more quickly than hydrogen. CO_2 production and energy expenditure are calculated with the rate of decline of the H-2 and O-18 isotopes (IAEA, 2008). Figure 6 illustrates the rate of decline of both isotopes over the monitoring period. The two lines represent a "*best fit*" (IAEA, 2008) between samples taken on days 1, 2 and 3, as well as 12, 13 and 14. As presented in the graph, besides a lower post-dose enrichment on day 0, the line representing the rate of decline of O-18 is slightly steeper than that of H-2.

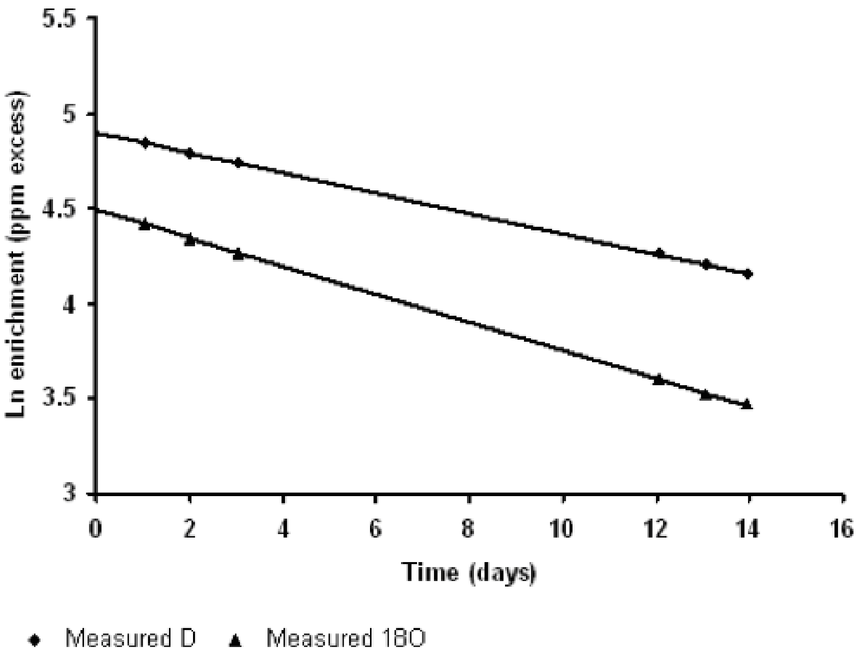

Figure 6: Natural logarithm (ln) to show decline of dose over time Christine Slater Source: data from Christine Slater (2016) in: "Using Isotopic Techniques to Accurately Assess Exclusive Breastfeeding".

The DLW technique is built on four assumptions (IAEA, 2009):

(1) Body water acts as a single compartment, steady-state pool, where incoming water is rapidly equilibrated and mixed throughout the pool. As mentioned in the context of the dose-to-mother technique, the dose of DLW given to the subject for measuring energy expenditure is mixed into the TBW, or equilibrated, within only a few hours.
(2) The H-2 and O-18 isotopes are removed from the body water only as H_2O and CO_2.

(3) The isotopes exit the organism "*only in isotopic equilibrium with body water*" (IAEA, 2009).

(4) H_2O and CO_2 that has left the body do not re-enter the body water compartment.

According to the IAEA (2009) report, assumptions (3) and (4) are both very robust, while adjustments must be made for (1) and (2). Regarding (1), adjustments must be made to account for the equilibration delay. This is considered to be around three hours, but deviations occur during pregnancy, shock and other cases where the metabolism and/or water turnover are altered. Regarding (2), other routes of excretion have been reported, but comprise less than one percent of total loss. This translates to a less than one percent change in energy expenditure and can thus be disregarded (IAEA, 2009).

4.3. Conventional Method

4.3.1. Direct Calorimetry

According to the Miller-Keane Encyclopedia (2003), direct calorimetry measures the amount of heat that a subject produces. More specifically, it measures the rate of heat production in a body as energy substrates are oxidized to water and carbon dioxide (IAEA, 2009). When applying direct calorimetry, the subject is enclosed inside a small chamber, a room calorimeter. This technique has also been used to evaluate impacts such as temperature or dietary changes on the energy expenditure (Rosado et al., 2013).

4.3.2. Indirect Calorimetry

Indirect calorimetry measures the metabolic rate by determining the amount of O_2 consumed, the amount of CO_2 eliminated from the body of a subject and the excretion of nitrogen via urine over a defined period of time. This method does not measure heat directly, but calculates it from the stoichiometric relationship when energy substrates react with oxygen, yielding carbon dioxide, water and energy (IAEA, 2009). All chemical energy comes from the oxidation of carbohydrates, proteins and fats. This method is based on the principle that the body hardly has reserves of O_2. Therefore, the amount of oxygen consumed reflects the oxidation of nutrients. As there is a fixed ratio of O_2 consumption and CO_2 production for oxidizing macronutrients, the concentration of inspired O_2 and expired CO_2, the energy expenditure can be derived with an equation, while also taking into account how much nitrogen is lost in urine during the test. It is also a useful tool for "*quantifying the utilization of macronutrients*" (Rosado et al., 2013).

The determinations of energy expenditure are based on the concentrations of water and carbon dioxide gases in inspired and expired air. Measurements are taken by using a mouthpiece, open or flow-through canopy, or an enclosed and flow-controlled room. Heat production is calculated with the measured concentrations (IAEA, 2009). As only the measurement technique is different, but the underlying principle and calculation process are the same, the IAEA (IAEA, 2009) refers to the DLW technique as a form of indirect calorimetry.

Rosado et al. (2013) state that this technique prevails as a "*gold standard*" in clinical settings and enables to deliver nutritional therapy that is customized to meet a patient's specific energy needs and nutrient uptake. Where indirect calorimetry is not available, researchers have recognized the use of particular predictive equations based on calorimetry studies of groups with similar clinical characteristics (Rosado et al., 2013). Other conventional methods include questionnaires, food records and metabolic rate measurement.

4.4. Comparison

4.4.1. Clinical vs. Free-Living Conditions

The foremost difference between (direct and indirect) calorimetric techniques and the stable isotope technique is that the former are suitable for clinical, or laboratory, use because the necessary equipment may strongly interfere with day-to-day activity. According to Ja (2005), open-circuit calorimeters use a mask, hood, canopy or chamber to collect the expired air. While mask, hood or canopy devices are applicable to short-term monitoring, long-term monitoring requires a room-based system. Calorimetric techniques can only measure the energy expenditure during a specified activity pattern under laboratory conditions, but does not allow to monitor everyday life (Ja, 2005). Rosado et al. (2013) point out that although indirect calorimeters have recently become more affordable and easier to operate, it is limited to its application in research as its conclusion is costly and takes too much time. Therefore, prediction equations are needed in clinical practice. Conversely, the stable isotope technique using DLW accurately measures total daily energy expenditure in free-living subjects under field conditions (Ja, 2005).

4.4.2. The Accuracy and Precision versus Cost Reduction Trade-off:

High accuracy demands the use of an indirect calorimeter, or the stable isotope DLW method. The indirect calorimetric technique requires sufficient resources to perform this method. If resources are limited and optimum precision is secondary, Ja (2005) suggests the use of flexible total collection systems and conventional non-calorimetric methods.

Rosado et al. (2013) point out that indirect calorimetry measures the net elimination rate of a substrate oblivious to the metabolic interconversions that may occur before the substrate disappears from its metabolic pool. Commonly, the substrate is directly oxidized, but in the case where gluconeogenesis, ketogenesis or lipogenesis rates are elevated, the direct translation of oxidation and substrate disappearance may not be valid. In those cases, other metabolic monitoring methods, such as the insulin clamp or the stable isotope turnover, i.e. the DLW, techniques step in to validate the conclusions made from the measurements made with indirect calorimetry (Rosado et al., 2013).

However, the DLW technique is connected to high costs and is very complex. As long as IRMS is the common technique to analyze O-18 enrichment in the samples, this characteristic will not change. FTIR can replace IRMS at an approximately tenfold increase in dosage, which is acceptable, but leaves room for cost reduction as an increase in dosage means higher costs of tracer enriched with H-2 and O-18 (IAEA, 2009). Therefore, where resources do not permit the use of calorific or stable isotope techniques in field applications or clinical practice, equations are drawn upon. Meanwhile, as touched upon previously, the DLW method is ideal for nutritional studies where field conditions and free-living subjects increase the quality of scientific findings. Consistently, Neilson et al. (2008) recognize that the DLW technique is optimal for studies of military nutrition. The study process hardly interferes with the natural behavior of the subjects. Calorific techniques using rooms or chamber calorimeters restrict the study subject to remain in the room for the duration of the measurement, i.e. several hours, even 24 hours if the purpose of the study requires such a time period. The requirements that DLW poses on the subjects are limited to urine (or saliva) samples. This also enables large population studies. The analysis of six samples per participant is much less time consuming than evaluating filled out logs or calorific records.

4.4.3. Equations versus the DLW Technique

As previously mentioned, it is impractical to use calorimetric methods to measure energy expenditure in clinical practice, therefore, researchers have recommended the use of equations. However, as Rosado et al. (2013) point out, the equations applied in common use internationally were developed predominantly in North America and Europe, which has led to distortions when applied in other parts of the world, especially on residents in tropical regions. Studies have shown that these distortions are due to different body compositions as well as environmental conditions (Rosado et al., 2013). Further, populations with strong prevalence of severe obesity pose challenges to selecting the weight to fill into the equation. The use of current weight leads to an overestimation of the energy expenditure, regardless of the equation applied, while using adjusted or optimal weight has resulted in an underestimation (Vander Weg et al., 2004).

Meanwhile, the DLW technique is applicable to individuals in the clinical practice and even in field conditions (IAEA, 2008). However, as previously mentioned, the DLW technique and the use of the IRMS instrumentation is very costly and requires advanced know-how. Under these conditions, the IAEA (2008) recommends the combination of the DLW technique with indirect calorimetry. The former is ideal to estimate the total energy expenditure, as well as the average daily energy expenditure under everyday conditions. The speciality of the latter is to measure "*basal or resting metabolic rate*" as movement in, or with, a calorimeter is severely limited and the conditions are artificial (IAEA, 2008).

In connection to the deuterium oxide dose-to-mother technique, another significant advantage of the DLW technique is that TBW measurement by isotope dilution forms part of the procedure of the DLW method. Therefore, body composition can be estimated simultaneously to TEE when applying the DLW method, according to the IAEA (2009). Body composition studies yield a number of findings that can illustrate a child or adolescent's quality of growth and support the development and evaluation of nutrition interventions and physical activity programs (IAEA, 2009).

4.5. Involvement of International Organizations

In 2001, FAO and WHO jointly published a report on energy requirements of children at the age of 2 to 18 years in developed and developing countries. The research is based on the DLW technique and validated heart rate methodology (FAO/WHO/UNU, 2001). The report referred to the DLW method as the ideal approach to measuring TEE of individuals in everyday living conditions. Furthermore, the report suggested that findings using other techniques to measure energy expenditure in children ought to be validated using the DLW method (FAO/WHO/UNU, 2001).

Currently, the IAEA is supporting projects to assess body composition and TEE in order to address a broad scale of nutritional priorities. Childhood obesity is one of these priority areas, beside acute severe malnutrition and HIV/AIDS in Latin America, Asia and Africa (IAEA, 2008).

5. Conclusion

The aim of this thesis was to shed light on malnutrition in general and specifically on vitamin A deficiency. This document is divided into 3 main areas:

- monitoring of breastfeeding
- vitamin A assessment
- measuring human expenditure and metabolic function

The first areas covered are the critical window of opportunity and the promotion of breastfeeding. Research shows that the first 1000 days after conception are vital to set basis for the child's future health. A well nourished mother has more capabilities to nourish a healthier child. In this regard, breastfeeding is a crucial point. Many organizations, such as WHO recommend exclusive breast feeding, because additional feeding is of the largest potential impact on child mortality and this can easily be prevented especially in developing nations, where food security is not always given. Seven million young children (under five) die annually from causes that are preventable and half of those deaths are newborns. Immediate breastfeeding right after birth would dramatically lower neonatal mortality rate. The monitoring and promotion of breastfeeding behaviour is tackled by many international organizations, in order to lower and eventually eliminate child mortality. To identify the need to promote breastfeeding among a population, several monitoring methods have been developed in the past. Conventional methods include test weighing and maternal expression or recall. Due to a number of drawbacks related to limited precision and reliability of the data and potential interference into natural patterns and practices, researchers have developed a method that uses stable isotopes to measure breast milk intake. Since infants are fed frequently during the day, the process of test weighing becomes very inefficient compared to the deuterium oxide dose-to-mother technique (IAEA, 2010), where measurement involves only a few steps from weighing mother and child twice each, offering the sample and taking post-dose samples from the saliva or urine of mother and child only six times each. There is much less risk of altering habitual feeding patterns during lactation, which attributes much higher data reliability to the isotopic method. Additionally, the specialists do not need to be there for the saliva sampling and cost cutting takes place because the samples are sent to their laboratories.

Considering the criterion of cost effectiveness, the isotopic technique may prove the most efficient method due to the possibility to monitor a range of other nutritional values, besides yielding precise results. Its precision and accuracy make direct supervision, as previously mentioned, and additional validation redundant, saving costs of both.

Another area of research of this thesis is the assessment of vitamin A. The isotope dilution technique can be evaluated in its efficiency and effectiveness. The data collected from these studies is then used to improve a society's nutritional status. The stable isotope dilution method includes the process of administering a dose of stable isotope-labeled vitamin A to the test subject and after that determining the isotope ratio in plasma at specific times. In order to predict the total body vitamin A pool size. The stable isotope dilution technique has favorably been used in the United States, Bangladesh, China, Guatemala, Philippines, Peru and Nicaragua in order to estimate the body vitamin A pool size of children and adults and further to evaluate the effectiveness and efficiency of national vitamin A interventions. In comparison to that it is not possible to trace vitamin A body storage in urine. Blood levels are not a sensitive reflector and do not deliver intermediate status and body storage. The most commonly used conventional methods in the field are clinical signs and symptoms, for example night blindness and conjunctival dryness. A different assessment of vitamin A via a conventional method is the serum retinol concentration. The assessment of this concentration requires a high performance liquid chromatography. This makes this application expensive, rarely available and technically demanding in developing countries. Many international organizations such as the IAEA and WHO sponsor and promote the assessment of vitamin A. The IAEA also further helps countries to implement food fortification and nutritional health plans to fight malnutrition.

The third part of this master thesis elaborates the understanding of the interaction between energy intake and energy expenditure, as well as body composition and its energy stores. This assessment helps to develop strategies to reduce obesity, either cut out for individuals or characteristic groups of individuals in a nation. In line with this, the measurement of energy expenditure enables the study of metabolic needs, dietary fuel utilization, as well as drug and emotional components to weight gain (Ja, 2005). Knowledge of these components helps to develop nutritional interventions with the highest possible efficiency and to make recommendations on nutritional and physical activity. A conventional method to gain data in this field is the direct calorimetry measurement, where the amount of heat that a subject produces is measured. Another non-nuclear technique is the indirect calorimetry, herein the metabolic rate is measured by determining the amount of O_2 consumed, the amount of CO_2 eliminated from the body of a subject and the excretion of nitrogen via urine over a defined period of time. This method does not measure heat directly, but calculates it from the stoichiometric relationship when energy substrates react with oxygen, yielding carbon dioxide, water and energy. In comparison to these two ways, there is an isotopic research that can be conducted to calculate human body energy expenditure. This technique is based on the principle that after intake of a dose of doubly labeled water ($^2H_2{}^{18}O$), the two isotopes of the water and oxygen molecules mix into the total body weight (TBW) and then exit differentially from the body. Deuterium ($^2H_2$) leaves the body as water, while ^{18}O leaves as both water (H_2O) and carbon dioxide (CO_2). The doubly labeled water (DLW) method is an accurate technique to validate other methods to determine the total energy expenditure of "free-living" subjects, as opposed to subjects tested under clinical conditions. The foremost difference between (direct and indirect) calorimetric techniques and the stable isotope technique is that the former are suitable for clinical, or laboratory, use because the necessary equipment may strongly interfere with day-to-day activity. Calorimetric techniques can only measure the energy expenditure during a specified activity pattern under laboratory conditions, but does not allow to monitor everyday life. Calorimeters are limited to their application in research as its conclusion is costly and takes too much time. Therefore, prediction equations are needed in clinical practice. On the other side, the stable isotope technique using DLW accurately measures total daily energy expenditure in free-living subjects under field conditions. However, the DLW technique is connected to high costs and is very complex. This method is, however, optimal for studies of military nutrition. The study process hardly interferes with the natural behavior and the only requirements are limited to urine (or saliva) samples. This enables large population studies. The analysis of six samples per participant is much less time consuming than evaluating filled out logs or calorific records. The IAEA is supporting projects to assess body composition and total energy expenditure, in order to address a broad scale of nutritional priorities. Childhood obesity is one of these priority areas, beside acute severe malnutrition.

After examining all different literatures, I come to the conclusion that nuclear techniques are superior. Looking at the results of this thesis and weighing the pros and cons, I draw the conclusion that nuclear techniques are the more expensive option but at the same time these techniques provide more detailed information and are less prone to deviations, since less human error can occur. This statement is valid for all three discussed fields (breast feeding, vitamin A and overweight).

However, in my point of view, education in the field of stable isotope techniques to the broad public is necessary. Many fear the methods because of their negative connotation, but in reality are harmless. Nevertheless, further research will lead us to a more thorough understanding and present us with the tools necessary to check and analyze the most suited method to trace vitamin A and other deficiencies. This will eventually enable us to help combat malnutrition and tackle child mortality at a lower price.

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