Stable Isotope Techniques to Develop and Monitor Nutrition Programmes

A. Introduction

The central role of nutrition to development is emphasized by the growing international awareness that the magnitude of malnutrition as a global health problem will prevent many countries from achieving the Millennium Development Goals. The urgent need for effective nutritional interventions is clearly indicated by the current global situation where - on the one hand - 170 million children are underweight and undernutrition is an important factor in more than half of all child deaths worldwide and - on the other hand – more than a billion adults are overweight. "The double burden of malnutrition", i.e., overlapping under- and overnutrition, results in a very heavy burden on health systems in countries where treatment of diet-related non-communicable diseases will be increasingly needed at the same time as undernutrition is still prevalent. In particular, infants and young children in resource poor settings are vulnerable to the devastating effects of undernutrition and poor health as demonstrated by the fact that 99 % of all young children dying in 2001 (10.6 million) lived in low and middle income countries and poor nutrition contributed to 1 out of 2 deaths.

Improved nutrition represents a high priority area as part of the development agenda in low and middle income countries. The IAEA assists Member States in their efforts to develop effective, evidence-based interventions to combat malnutrition in all its forms by nuclear techniques, in particular stable isotope techniques, and complements work by other UN agencies, in particular the WHO and UNICEF.

B. Stable Isotope Techniques in Nutrition

Stable, that is, non-radioactive isotopes of an element and the ability to measure these isotopes by mass spectrometry were first recognised in the 1920s (Aston 1927). Following a long history of use in research, stable isotopes are increasingly being used in the wider nutritional context. In part, this has stemmed from the inappropriateness of the use of radioisotopes in vulnerable population groups such as infants, children, and pregnant or lactating women. Secondly, the stable isotopes of carbon, hydrogen, nitrogen and oxygen have been commercially available and in relatively good supply in recent years. In addition, the sensitive, specific and precise analytical instrumentation for the measurement of stable isotopes, isotope ratio mass spectrometry (IRMS), is now more widely available. For example, the IAEA equipped three laboratories in Africa and Asia with IRMS dedicated to nutrition projects during 2007. However, due to the high establishment and maintenance costs of an IRMS facility there is an increasing interest in the use of Fourier transform infrared spectrometry (FTIR) for applications based on the use of deuterium. During the last few years, the IAEA has facilitated the establishment of a number of FTIR units in strategic locations in Member States, in particular in Africa, and contributed significantly to capacity building of personnel through various education and training initiatives.

There is a wide range of stable isotope techniques used in nutrition, however, the scope of this Annex is limited to an overview of three of the most widely used techniques with particular relevance to the development and monitoring of nutritional interventions globally. These techniques include the doubly labelled water technique of deuterium (^{2}H) and oxygen-18 (^{18}O) to assess total energy expenditure, the use of ^{2}H for the estimate of total body water and assessment of body composition as well as the deuterium oxide "dose-to-mother" technique to assess human milk intake in breastfed infants. The stable isotopes of hydrogen (^2H) and oxygen (^{18}O) are present in the body, food and water; about 0.015% of all hydrogen is deuterium while approximately 0.20% of all oxygen is ^{18}O. Thus, an adult man weighing 70 kg with 40 kg of body water contains almost 80 g ^{18}O water and about 6 g deuterium. Consequently, body cells are accustomed to molecules containing ^2H and ^{18}O at natural abundance levels.

C. The Nutrition Context: the Double Burden of Malnutrition

The 5th Report on The World Nutrition Situation (SCN 2004) estimated that childhood and maternal underweight alone are responsible for 138 million disability adjusted life years lost or 9.5% of the global burden of disease. However, many parts of the developing world are undergoing a rapid transition and the combined effects of industrialization, urbanization, economic development and globalization are having a significant impact on the health and nutritional status of these populations (Caballero and Popkin 2002). The main consequences of changes in diet and lifestyle patterns include an increase in chronic non-communicable diseases such as diabetes mellitus, cardiovascular disease, hypertension, stroke, osteoporosis, some cancers, and related conditions such as obesity. Obesity is a major public health problem in all industrialized countries and a burgeoning problem in developing countries and a key risk factor in the progression of chronic and non-communicable diseases. The WHO has projected increasing obesity rates projected over the next 20 years (WHO 2003).

The unique characteristics of the stable isotope techniques discussed in this Annex make these methods highly suitable for development and evaluation of interventions to address the urgent need to improve nutrition throughout the life cycle. These techniques are state-of-the-art methodologies to monitor changes in body composition, total energy expenditure and human milk intake in breastfed infants and thus provide tools to monitor the effects of altered diet and physical activity as well as interventions specifically targeted to improve infant nutrition. The IAEA has fostered the more widespread use of these techniques in Member States through support to national and regional nutrition projects via the Technical Cooperation Programme, and the development and transfer of technical expertise through numerous Coordinated Research Projects addressing priority areas in nutrition.

D. Body Composition Assessment

The most common approach in body composition assessment is to divide body mass into two compartments, fat mass and fat-free mass. The three commonly recognised primary body composition assessment techniques are densitometry, elemental analysis and the measurement of total body water. Densitometry involves the estimation of body density which has conventionally been made by underwater weighing. More recently, air displacement plethysmography has provided a simpler alternative. Both densitometry approaches are laboratory-based and therefore not suitable for use in field settings. Elemental analysis techniques, including total body *in vivo* neutron activation analysis and total body potassium analysis, are also limited in terms of wider application. Dual-energy x-ray absorptiometry is a widely used body composition method although not commonly used in field studies. The third primary body composition measurement technique is the assessment of total body water. The technique is based on the assumption that the water content of fat free mass is relatively constant (approximately 73.2% in adults) and that a negligible amount of water is associated with fat in adipose tissue.

Total body water assessment using stable isotope labels is the criterion method of body composition analysis and ideally suited for nutrition applications in field settings. Less exacting techniques, including anthropometry and bioelectrical impedance have been used in large nutrition interventions and population studies with validation against total body water in a representative sample.

D.1. Deuterium Oxide Dilution Technique for the Assessment of Body Composition

Deuterium oxide ($^{2}H_2O$) and ^{18}O-labelled water ($H_2{}^{18}O$) are both used for body composition assessment, however due to substantial differences in cost, the technique of choice is deuterium oxide dilution. The labelling of water molecules with deuterium enables the measurement of the dynamic character of body water. After consuming a dose of $^{2}H_2O$, the deuterium-labelled water is distributed throughout the body water pool and commonly reaches a steady state concentration in approximately 3-5 hours. The body water pool size, or deuterium dilution space, can be measured based on the concentration of deuterium oxide in body water and the exact dose of deuterium-labelled water consumed. Comparisons are made between pre-dose and post-dose samples of urine (IRMS only) or saliva (IRMS or FTIR).

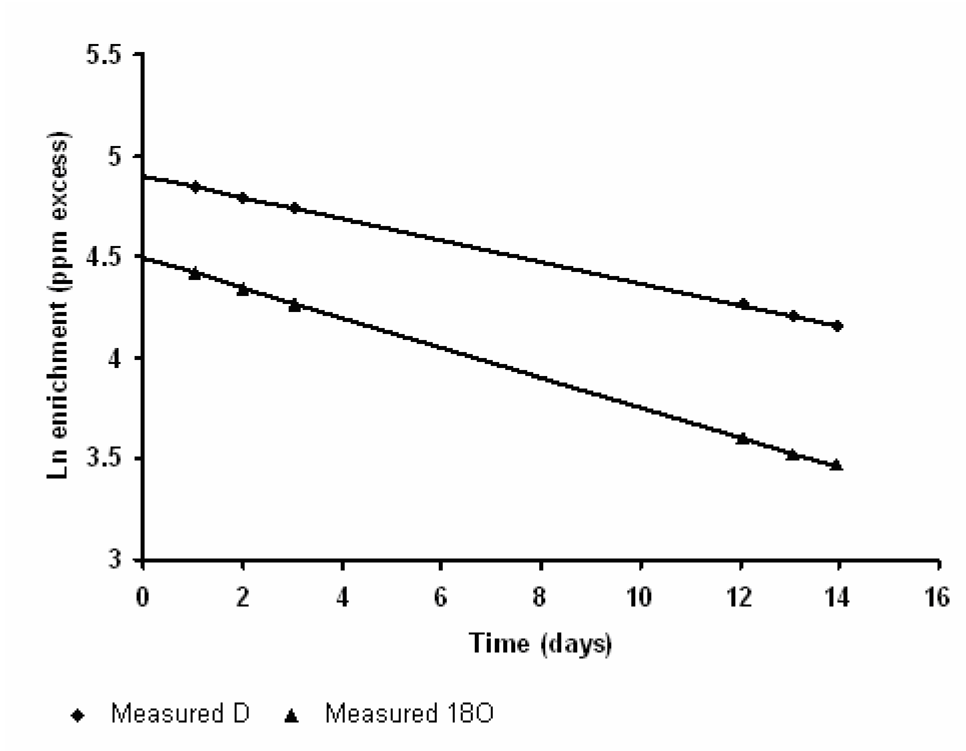
E. Assessment of Total Energy Expenditure

A number of methods, including food records, indirect calorimetry and the doubly labelled water technique have been used to estimate energy expenditure in humans. Whole room calorimetry is considered a gold standard for measuring 24-hour energy expenditure however, spontaneous physical activity (more recently termed non-exercise activity thermogenesis), and movement in general, is severely limited in such an environment. Energy expenditure measurement in room calorimeters is strictly controlled but the environment is very artificial.

E.1. The Doubly Labelled Water (DLW) Technique for the Assessment of Total Energy Expenditure

Unlike 24-hour energy expenditure measurement in a room calorimeter, the DLW technique is non-invasive, non-restrictive and enables the assessment of total energy expenditure under free-living conditions. Following the discovery of the application of the DLW technique for the determination of energy expenditure (Lifson and McClintock 1966), the technique has become the gold standard for the measurement of total energy expenditure. The technique is ideal for use in nutrition interventions in any field setting.

Using the DLW technique, average energy expenditure is normally assessed over a period of 14 days. Participants drink a dose of water containing the doubly labelled water which is rapidly distributed and mix with hydrogen and oxygen in body water. As the body expends energy, hydrogen and oxygen are lost from the body; oxygen is lost more quickly as it is present in carbon dioxide and water. The rate of decline of both isotopes in urine samples over the course of the study is used to calculate carbon dioxide production and energy expenditure. Figure II-1 illustrates the elimination of both isotopes over 14 days, the lines representing a ‘best-fit’ between samples measured at discreet time-points.



Ln = natural logarithm

FIG. II-1. Graph of stable isotope elimination in a multipoint DLW protocol.
Source: data from Christine Slater, UK

The best way to measure physical activity energy expenditure is to combine the gold standard DLW technique for the estimation of total energy expenditure (and average daily energy expenditure), with indirect calorimetry to measure basal or resting metabolic rate (Byrne et al. 2005). A recent report by the FAO/WHO on energy requirements (FAO/WHO 2001) is based on the DLW technique combined with appropriately validated heart rate methodology to establish energy requirements in children 2-18 years of age in both developed and developing countries. This report referenced the DLW technique as the optimal measurement approach for total energy expenditure of individuals in normal daily living conditions. The report also suggested that other methods of energy expenditure measurement in children should be validated against the DLW technique.

The IAEA is currently supporting projects on body composition assessment and total energy expenditure to address a wide range of priority areas in nutrition, including childhood obesity, acute severe malnutrition and HIV/AIDS in Latin America, Asia and Africa.

F. Nutrition during Early Life

Exclusive breastfeeding for 6 months, followed by the introduction of appropriate complementary foods and continued breastfeeding, as recommended by the World Health Organization (WHO 2001), are cornerstones in infant nutrition. However, only limited information is available on the quantities of human milk consumed and the time of introduction of other foods into the infants' diet, in particular in developing countries. The lack of information is, at least partly, due to the difficulties involved in measuring intake of human milk. By conventional technique, infants are weighed before and after each feed, "test weighing". This technique is obviously time consuming and can disturb the normal feeding pattern. In addition, in many settings, infants are nursed frequently - “on demand” - including during the night, resulting in severe practical limitations to the use of “test weighing”.

F.1. Deuterium Oxide “Dose-to-Mother” Technique to Assess Intake of Human Milk in Breastfed Infants

By using a stable isotope technique – “Deuterium oxide dose-to-mother- technique” - these practical problems can be overcome as the normal feeding pattern is not influenced and the average volume of human milk, consumed by the baby over a period of 14 days, is measured (Coward et al. 1982). Furthermore, the method is non-invasive as the dose of deuterium oxide is consumed orally by the mother and only samples of urine or saliva are collected for analysis. Briefly, after intake of deuterium oxide by the mother in a glass of water, deuterium is mixed with the mother’s body water and ingested by the baby via human milk (Fig. II-2). By measuring the disappearance of the stable isotope from the mother and its appearance in the baby (Fig. II-3) intake of human milk can be calculated. Information about whether the infant has consumed water from other sources than human milk can be obtained at the same time and the mother’s body water content can be measured. Based on total body water content, the mother’s fat free mass and fat mass can be estimated to provide important information about the nutritional status of the lactating mother.

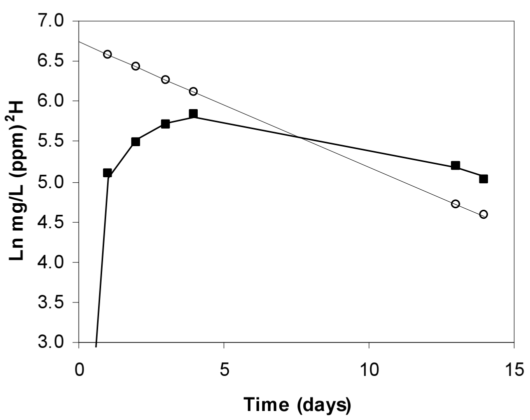
1. Mother drinks ^2H_2O

2. Baby consumes ^2H_2O in human milk

3. Saliva in both mother and baby is enriched with ^2H_2O

FIG. II-2. Dose-to-mother technique of assessing human milk intake.

(A)



(B)

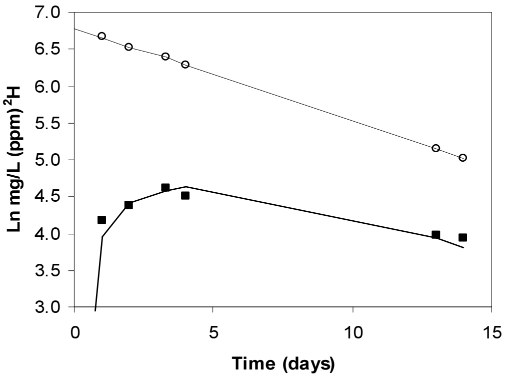


FIG. II-3. ^{2}H concentration in body water (saliva) collected from mother (○) and baby. (■)
Source: data from Les Bluck, UK

Differences in the deuterium concentration in saliva collected from two mother-baby pairs illustrated in Figure II-3 are evident. Modelling of the data reveal that baby A consumed considerably more human milk and only a small amount of water from sources other than human milk as compared to baby B who consumed less human milk than water from sources other than human milk.

The IAEA has supported projects using stable isotope technique to assess human milk intake in breastfed infants over a period of several years and thus contributed significantly to progress in this important area. The "dose-to-mother" stable isotope technique has been used in a wide range of field settings and experience with the technique as well as analytical equipment (FTIR) is now available in many countries.

G. Conclusions

The well-established stable isotope techniques outlined in this review have been utilised in a wide range of nutrition contexts, however there are significant opportunities for more widespread use. For example, the stable isotope technique to assess human milk intake can be used to optimise nutrition interventions for improving nutrition, health and well-being of infants and young children in developing countries and would thus contribute significantly to move the agenda forward. In addition, the use of stable isotopes such as deuterium and ^{18}O is crucial in the development and evaluation of nutrition interventions regarding body composition and energy expenditure, including in the determination of nutrition and physical activity recommendations compatible with healthy lifestyles for the global population.

For more information about the IAEA's activities in human nutrition, please visit the Nutritional and Health-related Environmental Studies Section's website:
http://www-naweb.iaea.org/nahu/nahres/default.shtm

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