New frontiers in science and technology: nuclear techniques in nutrition[1–3]

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ABSTRACT

The use of nuclear techniques in nutrition adds value by the increased specificity and sensitivity of measures compared with conventional techniques in a wide range of applications. This article provides a brief overview of well-established stable-isotope techniques to evaluate micronutrient bioavailability and assess human-milk intake in breastfed infants to monitor the transfer of micronutrients from the mother to the infant. Recent developments are highlighted in the use of nuclear techniques to evaluate biological interactions between food, nutrition, and health to move the agenda forward.

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INTRODUCTION

Bioavailability refers to the fraction of absorbed and used micronutrients and includes the storage of absorbed micronutrients. This concept is particularly important for some micronutrients (eg, nonheme iron and provitamin A carotenoids) because bioavailability varies widely depending on a number of factors. Stable (nonradioactive)-isotope techniques to assess micronutrient bioavailability have been developed over the past ≈20 y, and the application of these techniques has contributed significantly to our understanding of the importance of bioavailability in micronutrient nutrition. Because stable-isotope techniques do not expose the study population or investigators to potential health hazards related to radiation, studies in vulnerable population groups at high risk of developing micronutrient deficiencies are feasible. Over the past few years, information about dietary enhancers and inhibitors of nonheme iron absorption in infants and children have been made available. In addition, the use of stable-isotope techniques has contributed significantly to new data generated on nonheme iron bioavailability from iron compounds used in food-fortification programs. In vitamin A nutrition, the development of stable-isotope techniques to estimate body-pool sizes of vitamin A have provided new important information on the bioefficacy of provitamin A carotenoids and the influence of dietary composition, as well as the nutritional status of the consumer, on provitamin A bioavailability.

NONHEME-IRON BIOAVAILABILITY

From a methodologic point of view, the rapid incorporation of newly absorbed iron into a target tissue that can be sampled relatively easily (ie, erythrocytes) is a major advantage. Consequently, a stable-isotope technique to evaluate iron bioavailability has been developed on the basis of the incorporation of stable iron isotopes into erythrocytes 14 d after the administration of labeled test meals. This methodology was originally developed by investigators who used radioactive isotopes of iron. Usually, the incorporation rate of newly absorbed iron into erythrocytes is assumed to be relatively constant (≈80–90%). However, when the incorporation rate cannot be assumed to remain stable (eg, during pregnancy or in individuals infected with malaria), the incorporation of a stable isotope administered intravenously can be used to correct for changes in the incorporation rate. Considerable interindividual variation in iron bioavailability has been demonstrated that is largely due to differences in iron status in individuals, and therefore, paired comparisons are essential when the iron bioavailability from different foods or food fortificants is evaluated. With the use of a double-isotope technique [ie, the administration of 2 stable isotopes of iron (^{57}Fe and ^{58}Fe] on consecutive days], information about the iron bioavailability from 2 different test meals can be simultaneously obtained (1). Blood samples drawn at baseline and 14 d after administration are analyzed for ^{57}Fe and ^{58}Fe enrichment by thermal ionization mass spectrometry or high-resolution inductively coupled plasma mass spectrometry. Although the number of suitable mass spectrometers dedicated to nutrition remains limited worldwide, the application of this technique has been used in a wide range of settings with analysis of blood samples and dose preparations made in specialized laboratories in Europe and the United States. For example, studies have been performed in infants and young children in the Ivory Coast, Pakistan, Bangladesh, Jamaica, Peru, and Guatemala (2–8). The recently installed thermal ionization mass spectrometer in Bangalore, India, (A Kurpad, personal communication, 26 May 2010) will hopefully increase the application of this technique in Asia. As part of the efforts of the International Atomic Energy Agency to transfer technology and contribute to capacity building in the use of nuclear techniques in nutrition, a document on an iron stable-isotope technique is

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currently under preparation (K O'Brien and L Davidsson, personal communication, 27 May 2011).

The importance of iron-bioavailability data for setting dietary reference values was recently reviewed by Hurrell and Egli (9). These authors provided an excellent overview of dietary factors identified to influence iron bioavailability on the basis of earlier studies that used radioactive isotopes of iron, as well as more recent data on the basis of a stable-isotope technique. Inhibitors of iron bioavailability include phytic acid, polyphenols, and calcium, as well as some proteins, whereas ascorbic acid and muscle tissue (the meat factor) enhance iron absorption. More recently, the importance of nutritional deficiencies such as vitamin A and riboflavin deficiencies, infection/inflammation, and genetic disorders have received more attention, and there is clearly a need to further investigate the importance of these factors in different population groups. The identification of hepcidin as a key regulator of iron homeostasis (10, 11) highlights the need for future studies to investigate the role of this peptide and its increased expression during chronic inflammation and obesity on iron absorption. In addition, despite numerous studies, the influence of vitamin A, carotenoids, and nondigestible carbohydrates on iron absorption still remains largely unknown (9).

The concept of bioavailability is central to the development of food-fortification policies because the overall effect of a food-fortification strategy on the iron status of consumers will depend on the bioavailability of the iron compound used as well as the presence of inhibitors and enhancers of iron absorption in the diet. The importance of bioavailability data to optimize the effect of food fortification by selecting iron compounds with high relative bioavailability as well as by modifying the composition of the diet to increase iron absorption was highlighted in guidelines established by the World Health Organization and the Food and Agriculture Organization of the United Nations (12) and reviewed by Hurrell and Egli (13).

BIOAVAILABILITY OF PROVITAMIN A CAROTENOIDS

The development of stable-isotope techniques to evaluate changes in vitamin A body pools has contributed significantly to the development and evaluation of interventions on the basis of provitamin A carotenoids (14). Usually, deuterated retinol esterified to acetate with 4 or 8 deuterium atoms is administered followed by the analysis of deuterium enrichment in serum or plasma samples by gas chromatography–mass spectrometry (ie, the so-called paired stable-isotope dilution technique). The usefulness of stable-isotope techniques in vitamin A nutrition has been highlighted in a series of publications by the Vitamin A Tracer Task Force (15–17).

In particular, the paired stable-isotope dilution technique has been used to generate new data on provitamin A conversion factors. Tang et al (18) reported a mean conversion factor of 26.7 μg β-carotene:1 μg retinol for green and yellow vegetables consumed by Chinese school-age children, and Haskell et al (19) reported equivalency factors of β-carotene to retinol of 13.4 μg β-carotene:1 μg retinol for sweet potatoes, 9.5:1 for Indian spinach (*Basella alba*), and 6.3:1 for pure β-carotene in oil on the basis of studies in Bangladeshi men. An alternative stable-isotope method (ie, the extrinsic reference method) that is based on the administration of retinol labeled with a stable isotope (eg, deuterium or ^{13}C) at the same time as the food has also been used to evaluate the bioconversion of provitamin A carotenoids to retinol. For example, Edwards et al (20, 21) reported on the influence of dietary fat and food processing on conversion factors from carotenoids in carrots and spinach. Although some fat is needed for the absorption of carotenoids, fat does not seem to be a limiting factor when vegetables are consumed as part of a composite meal (22). Ribaya-Mercado et al (22) used the paired isotope dilution test to evaluate 3 amounts of fat (ie, 7, 15, or 29 g fat/d and 4.2 mg provitamin A carotenoids in the form of carotenoid-rich vegetables fed to Filipino schoolchildren). All groups of children had similar increases in serum carotenoid concentrations and a 2-fold increase in total body vitamin A.

With the use of a somewhat different approach, Tang et al (23) prepared intrinsically labeled vegetables by hydroponically growing carrots and spinach in deuterated water and used a reference dose of $^{13}C_8$-retinyl acetate for comparison. The intake of vegetables resulted in a conversion of 20.9 μg β-carotene:1 μg retinol and 14.8:1 for spinach and carrots, respectively. More recently, the intrinsic labeling technique was used to evaluate the bioconversion of provitamin A carotenoids in biofortified rice (ie, Golden Rice) (24). Compared with the reference dose, a mean conversion factor of 3.8 μg β-carotene to 1 μg retinol was reported in 5 adults. These new data highlighted the potential usefulness of biofortification to affect the vitamin A status. In general, animal and human studies that tested staple crops biofortified with provitamin A showed very good conversion rates of provitamin A to retinol, which demonstrated the feasibility of this agronomic technique (25). Plant sources of provitamin A are often overlooked in infant feeding strategies in developing countries. However, a simulation on the basis of an intake of 100 g orange-fleshed sweet potato/d to infants that used a 12 μg:1 μg conversion factor (26) resulted in a significant increase of vitamin A liver stores in infants (25).

Other applications of stable-isotope techniques in vitamin A nutrition have used a variety of methodologies. For example, the fecal excretion of extrinsically labeled [^{13}C]β-carotene was used in Indonesian children (27) and European adults (28). These studies used liquid chromatography coupled to a mass spectrometer. Although most studies have been based on extrinsically labeled provitamin A carotenoids, alternative labeling techniques that used [^{13}C]β-carotene from plant materials grown in a $^{13}CO_2$ environment have also been reported. Parker et al (29) used uniformly labeled [^{13}C]β-carotene from algae for metabolic studies in adults that used high-precision gas chromatography–combustion-isotope ratio mass spectrometry. Kale has also been labeled with $^{13}CO_2$ (30) and fed to adults (31, 32), followed by analysis with liquid chromatography coupled to a mass spectrometer.

From a methodologic point of view, it is important to recognize that the retinol response to provitamin A carotenoids in humans (33), as well as in animals (25), varies inversely with vitamin A status. There is an urgent need for additional, well-designed studies to evaluate the bioavailability of provitamin A carotenoids consumed in settings where vitamin A deficiency remains a public health problem in infants, children, and women of child-bearing age. In addition, more information is needed on the effect of nutritional interventions targeted at lactating women on the vitamin A nutrition of mothers and their infants. Accurate estimates of the transfer of retinol and provitamin A carotenoids via human milk are essential for a better understanding of the interactions between the maternal intake of retinol and provitamin A on the

infant vitamin A status. The use of stable-isotope techniques to estimate the total intake of human milk over 14 d in breastfed infants provides an excellent tool to monitor transfer of nutrients from mother to infant.

TRANSFER OF MICRONUTRIENTS FROM MOTHER TO INFANT VIA HUMAN MILK; STABLE-ISOTOPE TECHNIQUE TO ESTIMATE HUMAN-MILK INTAKE IN BREASTFED INFANTS

Exclusive breastfeeding for 6 mo followed by the introduction of appropriate complementary foods and continued breastfeeding, as recommended by the World Health Organization and the United Nations Children's Fund (34), are cornerstones in infant nutrition. However, only limited information is available on the quantities of human milk consumed to provide good estimates of micronutrient intakes in breastfed infants, in particular in populations at high risk of micronutrient deficiencies. The lack of information is, at least partly, due to the difficulties involved in estimating intakes of human milk. By the conventional technique, known as test weighing, infants are weighed before and after each feed. This technique is very time consuming and can disturb the normal feeding pattern. In addition, in many settings, infants are frequently nursed on demand, including during the night, which results in severe practical limitations of the use of test weighing.

With the use of a stable-isotope technique (the so-called deuterium oxide dose-to-mother-technique), these practical problems can be overcome because the normal feeding pattern is not influenced and the total volume of human milk, as well as the water intake from other sources than human milk, consumed by the baby over a period of 14 d can be estimated (35). This methodology is noninvasive because the dose of deuterium oxide is consumed orally by the mother and only samples of urine or saliva are collected for analysis. In brief, after the intake of deuterium oxide by the mother, deuterium is mixed with the body water of the mother and ingested by the baby via human milk. By measuring the disappearance of deuterium from the mother and its appearance in the baby, the intake of human milk can be calculated on the basis of a modeling procedure (35). Information about whether the infant has consumed water from other sources than human milk can be obtained at the same time, and the body water content of the mother can be measured. On the basis of total-body water estimates, the body composition of the mother (fat-free mass and fat mass) can also be calculated (36). 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 robust analytic equipment (Fourier-transform infrared spectrometry) is now available in many countries (37). When Fourier-transform infrared spectrometry is used for the analysis of deuterium enrichment, saliva should be collected from mothers and infants. In settings with access to isotope-ratio mass spectrometers, saliva or urine can be analyzed for deuterium enrichment (36).

RECENT DEVELOPMENT IN NUCLEAR TECHNIQUES IN NUTRITION

In addition to the previously mentioned well-established nuclear techniques in nutrition, developments in the use of ^{14}C and accelerator mass spectrometry (AMS) and the potential to use imaging techniques represent exciting approaches to address priority areas in micronutrient nutrition. A brief overview of applications in these areas is provided.

^{14}C and AMS

True tracer and tracee studies are preferably made with very small doses of the tracer to not perturb the tracee pool of the nutrient of interest. For vitamin A and β-carotene, this has been achieved with radioactively labeled ^{14}C-retinol and AMS (38). AMS was originally developed for carbon dating and, thus, is a highly sensitive, precise, and quantitative method for ^{14}C. This analytic technique has been applied to biomedical research (39); however, this application is limited because of the very high instrument and maintenance cost of an accelerator mass spectrometer (millions of US dollars). Nevertheless, this methodology was recently used in a field study in Zambia to assess the absorption, retention, and elimination of vitamin A in boys (40) after the administration of a very small dose (0.413 μg retinol as $^{11,12\text{-}14}C_2$-retinyl acetate; 25 nCi). The resulting dose of radioactivity was 2.1 mrem, which can be compared with a 6-h airline flight ($\approx$3 mrem) and the dose of a dental X-ray examination (20 mrem) (40). Other applications of the methodology included studies to determine the influence of supplemental vitamin A on the metabolism of ^{14}C-β-carotene (41), the conversion of β-carotene to retinol and metabolism to other retinoids (42), and the excentric cleavage of ^{14}C-β-carotene (43).

In addition to studies of vitamin A and provitamin A carotenoids, AMS and ^{14}C-labeled cyanocobalamin have recently been used to investigate the fate of vitamin B-12 during the passage through the gastrointestinal tract in humans (44). These unique studies provided new information on the variability of the degradation of vitamin B-12 and, thus, contributed to a better understanding of the metabolism of cyanocobalamin.

Imaging technique (positron emission tomography) for micronutrient kinetic studies

In nuclear medicine, imaging techniques are used to make the invisible visible in the diagnosis and treatment of diseases. Positron emission tomography (PET) is widely used in nuclear medicine and a rapidly increasing number of centers equipped with a PET, and cyclotrons for the production of radiopharmaceuticals are available worldwide (45, 46). To our knowledge, the possibility of using PET and radioactive isotopes of iron for studies of blood kinetics and organ uptake has been explored in studies by only one research group (47–49). These studies clearly showed the interesting potential of using PET in studies of iron metabolism. However, this application requires access to ^{52}Fe, which is an isotope with a half-life of 8.3 h and is not normally used in nuclear medicine applications and, thus, is not produced routinely. With wider global access to PET and cyclotrons, the feasibility of using this technique to address important areas such as iron metabolism and infectious diseases (eg, malaria and HIV) should be explored.

CONCLUSIONS

Nuclear techniques provide excellent tools to address priority areas in micronutrient nutrition. In particular, the contribution of stable-isotope techniques to better understand the micronutrient bioavailability and its crucial role in the development of effective

nutrition interventions cannot be underestimated. The usefulness of other well-established stable-isotope techniques, such as isotope-dilution techniques, to assess human-milk intake in breastfed infants and the body composition of lactating mothers (as well as the body composition of any other population group of interest) should be further explored to evaluate the transfer of micronutrients from mothers to infants as well as studies of body composition to evaluate interactions between overweight and obesity, inflammation, and micronutrient nutrition. Finally, the use of imaging techniques in nutrition should be considered in areas where making the invisible visible is crucial to move the agenda forward.

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