Nutritional and Health-Related Environmental Studies (NAHRES)

Optimising nuclear techniques to assess vitamin A status and the risk of excess vitamin A intakes due to multiple vitamin A programmes

Brief summary

The proposed CRP will contribute to a better understanding of the appropriate use of the stable isotope dilution technique to assess vitamin A status across the continuum from deficiency to toxicity in children under the age of 5 years. The findings will provide guidance for programme managers and public health nutrition policy makers to improve the evaluation of their vitamin A programmes, in particular in countries where multiple vitamin A interventions are in place, and enable them to improve national nutrition strategies and plans.

The combination of malnutrition, including micronutrient malnutrition, and infectious diseases is the most prevalent and preventable public health problem in the world; responsible for millions of deaths annually, particularly in infants and children. Infections affect nutritional biomarkers making it difficult to assess the real magnitude of some nutritional problems, for example vitamin A deficiency. Vitamin A is involved in numerous physiological processes essential for normal growth and development, the immune system, the visual system, and other functions in the human body. Vitamin A deficiency is a major nutritional concern in low-resource households in low-income countries. Vitamin A fortified foods such as sugar, margarine, vegetable oil, milk, and wheat flour as well as micronutrient powders and supplements, have been used as a complementary approach. The potential risk of excess vitamin A intake is increased due to a lack of coordination to avoid overlap of intervention coverage. Additionally, assessing vitamin A status, and the effectiveness of government interventions, is challenging in settings where infectious diseases and micronutrient deficiencies are endemic, as in most low-income countries.

The isotope dilution technique, which will be used in this CRP, is among the most accurate techniques for assessing total body vitamin A pool size in individuals. However, it is not known whether the method is valid under conditions of hypervitaminosis A, inflammation and selected micronutrient deficiencies. Therefore, this CRP will address methodological issues in the application of the stable isotope dilution technique to accurately determine vitamin A stores in children and thus, provide important new knowledge on optimising the stable isotope dilution technique for the assessment of vitamin A status. The CRP will be complementary to a grant of the Bill & Melinda Gates Foundation that assesses the risk of vitamin A toxicity due to large scale food fortification and other interventions with the University of Newcastle as grantee.

Background

Malnutrition and infectious diseases

Adequate nutrition is integral to improving and sustaining the health and wellbeing of children early in life. Undernutrition (namely, fetal growth restriction, suboptimum breastfeeding, stunting, wasting, and deficiencies of vitamin A and zinc) is estimated to cause 45% of all deaths among children under 5 years of age [1]. From a life-cycle perspective, the most crucial time to meet a child's nutritional requirements is in the 1,000 days including the period of pregnancy and ending with the child's second birthday. During this time, the child has increased nutritional needs to support rapid growth and development, is more susceptible to infections and is totally dependent on others for nutrition, care and social interactions [2].

A cornerstone of adequate nutrition is micronutrient intake. An estimated two billion people worldwide suffer from micronutrient deficiencies of essential vitamins and minerals (including trace elements), primarily iodine, iron, vitamin A and zinc [3]. Micronutrient malnutrition contributes substantially to the global burden of disease and is responsible for a wide range of non-specific physiological impairments, leading to reduced resistance to infections, metabolic disorders, and delayed or impaired physical and psychomotor development [4]. In high risk groups such as

young children and women, micronutrient deficiencies are widespread and have substantial adverse health effects on survival and development.

The combination of malnutrition, including micronutrient malnutrition, and infectious diseases is the most prevalent and preventable public health problem in the world; responsible for millions of deaths annually, particularly in infants and children [5].

Severe malnutrition – including micronutrient deficiencies – often masks symptoms and signs of infectious diseases, which makes prompt clinical diagnosis and treatment very difficult. Another issue is that infections affect nutritional biomarkers making it difficult to assess the real magnitude of some nutritional problems. That is for example the case of vitamin A. Vitamin A deficiency (VAD) is defined to be of public health importance if 15% or more of a defined population has a plasma retinol concentration of less than 0.7 µmol/L. However, circulating concentrations of plasma retinol are reduced by infections and in such situations plasma retinol concentration is not a good indicator of vitamin A status. Infections are accompanied by an acute phase response, and plasma acute-phase proteins can indicate the severity and duration of an infection. Retinol binding protein (RBP), the protein that binds to retinol when circulating in plasma, is a negative acute phase protein and therefore, during infection, RBP will fall and retinol concentrations with be depressed for an undetermined length of time [6]. Acute-phase proteins such as C-reactive protein (CRP) increase within the first 6 hours of infection, and reach their maximum concentrations within 24 to 48 hours. However, a1-acid-glycoprotein (AGP) rises more slowly, with maximum concentrations 2 to 5 days after infection.

Vitamin A deficiency and national vitamin A programmes

Vitamin A (retinol and retinal) – an essential nutrient needed in small amounts – and its derivative retinoic acid (RA) are involved in numerous physiological processes essential for normal growth and development, the immune system, red blood cell production, the visual system, and other functions in the human body [7]. The isomer 11-*cis* retinal is crucial for vision, whereas RA, which is a transcriptional regulator exerts most of the physiological functions of vitamin A (e.g. in embryonic development, reproduction, cell growth, differentiation and immunity).

VAD is a major nutritional concern in low-resource households in low-income countries located primarily in the Africa and South-East Asian regions, with an estimated 200 million children and 19 million pregnant women globally who are vitamin A deficient [7]. Usually, VAD develops in an environment of ecological, social and economical deprivation, in which a chronically deficient dietary intake of vitamin A coexists with severe infections, such as measles, and frequent infections causing diarrhoea and respiratory diseases that can lower intake through depressed appetite and absorption, and deplete body stores of vitamin A through excessive metabolism and excretion [8, 9]. The consequent “synergism” can result in the body’s liver stores becoming depleted and peripheral tissue and serum retinol concentrations decreasing to deficient levels, raising the risks of xerophthalmia, further infection, other VAD disorders and mortality.

VAD impairs numerous functions and, as a result, can lead to many health consequences, to which infants, young children and pregnant women appear to be at greatest risk. Xerophthalmia is the most specific VAD disorder, and is the leading preventable cause of blindness in children throughout the world [10]. Night blindness often appears during pregnancy, a likely consequence of pre-existing, marginal maternal vitamin A status superimposed by nutritional demands of pregnancy and inter-current infections [11]. Anaemia can result from VAD in children and women, likely due to multiple apparent roles of vitamin A in supporting iron mobilization and transport, and hematopoiesis [12]. Pre-existing VAD appears to worsen infection [13] and vitamin A supplementation has been shown to reduce the risk of death in 6–59 month old children by about 23–30% [14-16]; hence, VAD is associated with a higher mortality risk. All infants are born with low stores and depend on vitamin A from breast milk to initially accumulate and maintain adequate stores. Infants of vitamin A depleted women are at greater risk of becoming vitamin A deficient early in life.

Three types of community interventions can reduce VAD in affected populations. Improving the availability and intake of vitamin A through dietary diversification should be viewed as an activity for all communities in order to enhance the overall nutritional status of the population. A second approach to increasing the dietary intake of vitamin A is through fortification of a staple food or condiment with vitamin A. This has been the primary strategy for reducing VAD in Central and South America, where sugar began to be fortified with vitamin A three decades ago [17]. Thirdly, the most widely practiced approach to controlling VAD in most high risk countries is the periodic delivery of high-potency supplements, containing 200,000 IU of vitamin A, to preschool-age children (<5 years), with half this dose given to infants 6–11 months of age. In the past decade, vitamin A supplementation gained momentum as it was added to the annual Expanded Programme for Immunization (EPI) visits, especially within the poliomyelitis eradication campaign, that has since continued as national child health week campaigns during which high-potency vitamin A is distributed twice yearly in many countries. Many high-risk countries have also adopted the WHO policy of supplementing mothers with a 200,000 IU oral dose of vitamin A within six weeks after delivery to enrich their breast milk content of vitamin A, although in practice coverage remains quite low [7].

Vitamin A fortified foods such as sugar, margarine, milk, wheat flour, vegetable oil, corn flour and instant noodles as well as micronutrient powders and supplements, have been used as a complementary approach in low-income country settings, although there is rarely coordination to avoid overlap of intervention coverage. Additionally, assessing vitamin A status, and the effectiveness of government interventions, is challenging in settings where infectious diseases and micronutrient deficiencies are endemic, as in most low-income countries.

Vitamin A excess and upper level of intake (UL)

It is fundamental that the outcome of a fortification programme is to ensure that it raises the vitamin A status of deficient individuals yet at the same time does not lead to harmful or excessive intakes [18, 19]. For example, simulations using dietary data from Uganda and Cameroon predict that fortification of multiple food vehicles could result in >20% of young children having vitamin A intakes above the upper tolerable intake level (UL), if fortification levels were not adjusted to account for the effects of the other programmes. For safety considerations, combined continuous intakes from fortification, supplementation, and vitamin A or provitamin A-rich foods should not exceed the UL, based on a no-observed-adverse-effect level (NOAEL) or when unavailable, on the lowest-observed-adverse-effect level (LOAEL) [20]. Due to lack of data among children to link chronic vitamin A intake to markers of toxicity, the ULs set by the United States Institute of Medicine for children >1 year of age are extrapolated from those of adults (the UL for children <1 year was set using a LOAEL derived from 4 case reports in 1965). Thus, there are questions about whether these extrapolated values are appropriate. Data are needed to define an appropriate UL for children, to enable accurate assessment of the risk of excess vitamin A intake from overlapping vitamin A programmes.

There are clear concerns about inadvertent chronic excessive retinol intakes due to frequent supplementation combined with concurrent use of fortified staple foods, micronutrient powders and voluntarily fortified commercial products [19, 20]. Sub-toxic liver vitamin A concentrations (>1µmol/g liver) have been reported in Nicaraguan school children one year after implementation of sugar fortification [21]. Chronic excessive vitamin A intake can create liver abnormalities including perisinusoidal fibrosis and hypertrophy and hyperplasia of Ito cells, which are key effector cells in the evolution of fibrosis and cirrhosis [20, 22].

Measurement of vitamin A status – methodological challenges

To determine whether intervention programmes place some individuals at risk, sensitive biomarkers of vitamin A status are needed to evaluate the effectiveness and safety of vitamin A interventions across the full spectrum of vitamin A status from deficient to toxic, especially since serum concentrations of retinol show very little responsiveness to changes in vitamin A status [21, 23]. The isotope dilution technique is a powerful method that has been applied to estimate low to adequate vitamin A status in populations, including young children [24], and it has

also been used successfully in apparently healthy populations in low-income countries to assess the efficacy of vitamin A interventions and to estimate vitamin A requirements [21, 25, 26]. However, the technique has not been sufficiently validated under conditions of high vitamin A stores.

Determination of hypervitaminosis A in Rhesus monkeys indicated discrepancies between measured and predicted total body stores, largely due to the variability in the fraction of dose in plasma, suggesting that when vitamin A stores are high, exchange between the liver and plasma pools is compromised [27]. Specifically, there is concern that the dose (retinol labelled with deuterium or carbon-13) may not fully mix with the vitamin A that is stored in liver stellate cells or in other extrahepatic storage pools with slow turnover rates when vitamin A stores are very high. It was also shown that inflammation, as well as micronutrient deficiencies (e.g. iron deficiency) can influence mean plasma vitamin A specific activity (fraction of dose in plasma retinol/mass of plasma retinol) [28, 29]. Iron deficiency and inflammation in rat models inhibit mobilization of vitamin A stores and decrease the absorption and utilization of vitamin A [28, 29]. A reduction in dose absorption may lead to an overestimation of the true total body stores of vitamin A. These conditions could affect the accuracy of estimates of total body vitamin A stores. In addition, the use of different isotope labels for the vitamin A and dose sizes might influence the accuracy of results on total body stores of vitamin A, because of differential mass isotopic effects. Methods that use the heavier molecules to label the vitamin A, such as $^{2}H_{4}$ and $^{2}H_{8}$ or $^{13}C_{10}$ will have more potential mass isotopic effects *in vivo* than a method that uses $^{13}C_{2}$. However, dose size may cause more biological variation in human vitamin A assessment [6].

Due to potential problems in detecting toxic liver stores using the stable isotope dilution technique, it is important to perform concurrent comparisons of additional biomarkers alongside to gain more information as to how the method works under conditions of hypervitaminosis A. Markers of excess intake such as fasting retinyl esters, retinol-to-retinol-binding protein ratio, polar retinoid metabolites, as well as liver enzymes of fibrogenesis could be used to aid the detection of excess intake.

Lastly, existing deuterium-vitamin A isotope dilution techniques cannot be used in infants because they rely on the 'Olson' equation to quantify vitamin A pool size. The 'Olson' equation requires information on the fractional catabolic rate, which is not yet known for infants, and provides a precise estimate of body vitamin A pool size based on the measurement of specific activity of the labelled vitamin A in plasma at around 20 days after an oral dose is given. A new equation has been developed by Professor Mike Green in collaboration with the research team of Dr. Lietz in adults, which measures total body vitamin A pool size at 3 days after an oral dose of ^{13}C-labelled retinol. At the same time, the new method is more sensitive than existing deuterium-retinol isotope dilution methods, and allows the administration of very small physiologic doses to infants, therefore providing the opportunity to measure plasma specific activity of $^{13}C_{10}$-retinol in a much smaller volume of plasma [30]. However, it is not clear whether retinol specific activity in plasma and stores equilibrates in infants at 3 days as it does in adults. Therefore, validation of this novel isotope dilution technique to assess vitamin A status in infants is required.

The overall goal of the proposed CRP is to contribute to validating methods to assess vitamin A status and the risk of vitamin A toxicity due to large scale food fortification and other interventions in countries with national programmes to reduce vitamin A deficiency. Populations in low income countries that are exposed to multiple vitamin A interventions also have high rates of infection, inflammation and other nutrient deficiencies. Stable isotope methods will be used to evaluate vitamin A status and infant's breast milk intake to determine vitamin A intake in breastfeeding children. However, a better understanding of the accuracy and application of the isotope technique to estimate total body vitamin A stores in individuals with hypervitaminosis, infection, inflammation and selected micronutrient deficiencies is urgently needed. The proposed CRP will therefore provide guidance and recommendations on the use of the isotope dilution technique to assess vitamin A status across the continuum from deficient to sub-toxic and toxic status in the context of high rates of infection, inflammation and/or selected micronutrient deficiencies.

Analytical techniques to be used

To evaluate the impact of large-scale vitamin A fortification and other vitamin A interventions on vitamin A status of children under the age of 5 and to assess methodological issues, the stable isotope dilution technique will be used. This technique provides a quantitative estimate of total body vitamin A pool size and estimated liver vitamin A concentration, based on the assumptions that 90% of the total body vitamin A is stored in the liver and the weight of the liver has a fixed relationship to body weight at different ages [31]. An oral dose of vitamin A labelled either with the stable isotope of hydrogen (deuterium, ^{2}H) or with the stable isotope of carbon (^{13}C) will be administered to subjects, a blood sample will be collected after the dose has mixed with endogenous vitamin A and the plasma isotopic ratio of labelled to unlabelled vitamin A will be measured, using either liquid chromatography coupled to mass spectrometry (LC/MS/MS), gas-chromatography-mass spectrometry (GC-MS) or gas-chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) depending on the label. The total amount of vitamin A in the body will be estimated using a prediction equation.

To assess vitamin A intake from breast milk, the amount of breast milk taken in by the infant will be measured using the well-established deuterium oxide 'dose-to-mother' technique. Deuterium enrichment of urine and/or saliva samples will be analysed by Fourier Transformed Infrared Spectrometer (FTIR) or by isotope ratio mass spectrometry (IRMS).

Overall objective

To provide new knowledge and evidence on the application of the isotope dilution technique to assess vitamin A status and the risk of excess vitamin A intake in children under the age of 5 years, where large scale food fortification and other interventions are in place to reduce vitamin A deficiency.

Specific research objectives (purpose)

Obtain new information on the validity of the stable isotope dilution technique to assess total body stores of vitamin A across the continuum from deficient to sub-toxic and toxic status in the context of selected micronutrient deficiencies, inflammation and infection.

Expected outputs

1. New data on the effects of high vitamin A consumption from exposure to multiple vitamin A interventions on total body vitamin A pool size in children under the age of 5 and on vitamin A content in breast milk.
2. New data on the accuracy and feasibility of the stable isotope dilution technique to assess excessive vitamin A body pools.
3. New data on the impact of clinical/subclinical inflammation and selected micronutrient deficiencies on the vitamin A status of children under the age of 5 and the accuracy of the stable isotope dilution technique.
4. New data on the effect of different isotope labels and dose sizes on the accuracy of the stable isotope dilution technique to assess total body vitamin A stores.
5. New data on the 3-day isotope dilution method for use in infants and young children.
6. Publications in the form of scientific reports and peer-reviewed papers and conference presentations.
7. Technical brief or document to guide the application of stable isotopic methods for measuring total body stores of vitamin A.

Expected outcome

The appropriate use of the stable isotope dilution technique to assess vitamin A status across the continuum from deficiency to toxicity will provide guidance for programme managers and public health nutrition policy makers to optimize the evaluation of their vitamin A programmes, in particular in countries where multiple vitamin A interventions are in place, and enable them to improve national nutrition strategies and plans.

Proposal submission forms

Research institutions in Member States interested in participating in this CRP are invited to submit proposals directly to the Research Contracts Administration Section (NACA) of the International Atomic Energy Agency: Official.Mail@iaea.org or to Ms Cornelia Loechl: C.Loechl@iaea.org
The forms can be downloaded from http://www-crp.iaea.org/html/forms.html. For more information about research contracts and research agreements, please visit our web-site: http://www-crp.iaea.org/html/faqs.html.

Deadline for submission of proposal

Proposals must be received **no later than 10 January 2015**. Transmission via Email is acceptable if all required signatures are scanned.

For additional information, please contact:

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