Nutrition and Health-Related Environmental Studies (NAHRES)

Optimising nuclear techniques to assess vitamin A status in population surveys – from deficiency to excess (Phase II)

Brief summary

Vitamin A deficiency (VAD) remains a leading cause of childhood blindness and is a major contributor to infectious disease morbidity and mortality among pre-school children. As a response to the population risk, supplementation as well as fortification with vitamin A (VA) are in place in many low-income countries, but unfortunately there is rarely any coordination to avoid overlap of interventions or to monitor long-term effects. Despite VA deficiency still being widespread in some communities, there are concerns about inadvertent chronic excessive VA intakes in other population groups.

Serum retinol is widely used as a biomarker for VAD, but due to its tight homeostatic control, it cannot reflect changes in status when VA stores are adequate or excessive. Assessment of population VA status and evaluation of VA programmes require a biomarker that can estimate risk of VA deficiency as well as excess. The retinol isotope dilution (RID) technique, a nuclear technique, is the only method that can quantitatively measure VA status over the entire spectrum from deficiency to excess without the need for a liver biopsy.

The priorities for the proposed CRP are to optimise the RID for use in population surveys and to further optimise the field procedures, calculations, and interpretations for use by non-experts. This will support the monitoring of vitamin A programmes in the context of a dual risk of VA malnutrition - deficiency as well as excess.

Background

Vitamin A deficiency (VAD) remains a leading cause of childhood blindness and is a major contributor to infectious disease morbidity and mortality among pre-school children. VAD develops in an environment of ecological, social, and economic deprivation, in which a diet chronically deficient in VA coexists with frequent infections that lead to decreased nutrient uptake and increased nutrient loss. VAD was estimated to affect 200 million pre-school children and 19 million pregnant women globally in the years 1995-2005 [1]. This corresponds to one third of pre-school children and 15% of pregnant women in low-income countries at risk of VAD [1].

An update from 2015 focusing on attributed mortality showed significant progress in reducing the number of deaths attributable to vitamin A deficiency [2], particularly in South East Asia, Oceania and Latin America. Vitamin A deficiency remains a public health problem of concern in Sub-Saharan Africa and South Asia, where is it responsible for an estimated 100 000 deaths every year [2]. However, the data emphasizes that micronutrient deficiency is not a static problem, and the need for vitamin A interventions must be continuously evaluated and adapted to the needs. This effort relies on good monitoring systems and reliable data.

As a response to the population risk, VA supplementation programmes with biannual distribution of high doses of VA to children below 5 years of age have been implemented for decades in many low-income countries. VA-fortified foods such as sugar, dairy products, flour, vegetable oil, as well as micronutrient supplements, have been used as a complementary approach, but unfortunately there is rarely any coordination to avoid overlap of interventions or to monitor long-term effects. Despite VA deficiency still being widespread in some populations, there are concerns about inadvertent chronic excessive VA intakes due to multiple overlapping interventions coupled with increasing access to foods naturally rich in pro-VA [3, 4]. This dual risk of deficiency and excess calls for a biomarker that can accurately assess VA status across the continuum from deficiency to toxicity to guide programme evaluation [5].

Vitamin A excess and toxicity

VA is stored in the liver and the body has no mechanism to actively excrete excess intake of preformed VA. When VA stores are high, conversion of pro-VA is downregulated, but excessively consumed preformed VA is accumulated in the liver. Chronic excessive VA intake can lead to liver fibrosis and cirrhosis [4, 6]. Excess VA intake may also interfere with bone mineralisation [7]. The risk of excess VA intake has not received much attention until recently, and there is a need for more data to better define toxicity levels for liver VA concentration to assist evaluation of vitamin A programmes and interventions.

Toxicity cut-off values from 1 to 10 µmol/g liver have been suggested [8, 9], but there is no consensus, and the upper cut-off value must be calibrated against a variety of toxicity markers indicating clinical concern. It is of paramount importance to define cut-offs for deficiency as well as toxic hepatic VA concentrations to optimize the RID method for use in population surveys.

Measurement of vitamin A status

Serum retinol is widely used as a biomarker for VAD, but due to its tight homeostatic control, it cannot reflect changes in status when VA stores are adequate or excessive. In addition, serum retinol is suppressed by inflammation, thus limiting its validity as a biomarker in populations with a high burden of infections. This can to some extent be corrected by simultaneous measurement of and adjustment for inflammatory markers [10].

The retinol isotope dilution (RID) method, a nuclear technique, is the only method that can quantitatively measure VA status over the entire spectrum from deficiency to excess without the need for a liver biopsy [9]. A dose of labelled VA is administered in the mouth of a study participant and allowed to mix with the body VA pool (typically 1 to 3 weeks) [11]. After the mixing period, a blood sample is taken and plasma isotopic ratio of labelled to non-labelled retinol is measured using mass spectrometry. The isotope ratio enables quantitative estimation of total body VA stores (TBS).

There are different ways to calculate TBS. The Olsen equation and the mass-balance equation both use fixed values for the assumptions or coefficients and provide an accurate quantitative estimate of mean TBS for groups of individuals. More accurate results can be achieved at individual level by the application of newer and more advanced mathematical approaches using compartmental modelling. TBS can be estimated for individual children using population-specific coefficients (FaS), which are developed taking the vitamin A kinetics under different conditions into consideration, and the retinol

isotope dilution equation. The compartmental modelling approach potentially gives superior accuracy at individual level, and upon generation of suitable composite coefficients for different population groups, is suitable for individuals at all ages and nutritional statuses. The FaS values are generated based on kinetic studies in a small group of individuals at similar age/vitamin A status/nutritional status.

The RID method was recently explored and applied to assess VA status from deficiency to excess in different populations and in the context of inflammation. The research project concluded that the RID can accurately assess changes of VA stores even when stores are high. However, a few research questions remain to be confirmed or addressed, above all, the extent to which the method is affected by inflammation at the time of dosing. In addition, markers of VA toxicity need to be better studied and clear cut-offs for TBS and hepatic VA concentration be defined.

What will the proposed follow-up CRP add?

Assessment of population VA status and evaluation of VA programmes require a biomarker that can estimate risk of VA deficiency as well as excess. The priorities for the proposed second phase of the CRP aligned herewith are to optimise the RID for use in population surveys and streamline the field procedures, calculations and interpretations suitable to apply for non-experts with an appropriate link to a laboratory with the necessary analytical capacity. Specific priorities of the proposed CRP include:

- **Cut-offs** to define deficiency and excess of TBS and/or liver concentration of VA in different age groups to use at population level must be established before the method is suitable for population surveys.
- The quantitative impact of inflammation at the time of dosing on dose absorption needs to be further studied to make decisions on assessment of **inflammation** and/ or exclusion of individuals who have evidence of inflammation at the time of dosing.
- While generic values of the composite coefficient **FaS** have been published [12] for use in compartmental model-based calculations, it would be helpful to generate values for special population groups such as pregnant and lactating mothers, obese individuals, individuals with low VA stores and iron deficiency.
- Simplifications to the field protocol and calculations: There are some potential ways to **simplify** the field protocol, however most need to be further studied and pilot tested. This includes dose administration in capsules, number of samples and timing and amount of blood for different analytical setups. In addition, a simple calculation tool for non-modellers would be very helpful for the application of the method at larger scale.
- Clarification of the required level of accuracy to calculate unbiased proportions of VA deficiency, adequacy and excess.
- Testing the feasibility of the integration of the RID in a population survey. A survey suitable for integration at scale could be identified to pave the road for future integrations through a supported pilot study.

Requirements for this CRP

This CRP will focus on adaptations and tools needed for successful scale up of the RID. Thus, research groups are encouraged to submit proposals addressing one or more of identified priorities. Sample size considerations and a timeline for field work must be included. Specific requirements include:

- Experience with field aspects of the RID (dosing, blood sampling)
- A mechanism to follow study participants with a negligible loss to follow up over a period of one month
- Experience with assessment of dietary intake of vitamin A (including supplements and fortified foods)
- An established collaboration with an expert laboratory will be an asset

Overall objective

The overall objective is to provide new knowledge on how to use the retinol isotope dilution technique in larger population surveys to assess vitamin A status from deficiency to excess

Specific objectives

1. To establish cut-off values of the RID outputs to define vitamin A deficiency and excess
2. To explore how the retinol isotope dilution (RID) technique is affected by inflammation at the time of dosing
3. To generate coefficients for accurate calculation of vitamin A status in various population groups
4. To examine how the existing methodology can be simplified to suit population surveys without loss of accuracy

Expected outcome

To contribute to improved monitoring of vitamin A programmes and national nutrition strategies by enabling assessment of vitamin A status in population surveys - from deficiency to excess.

Expected outputs

1. New data on cut-off values to define vitamin A deficiency and excess
2. New data on the effect of inflammation at the time of dosing on dose absorption and the effect on the vitamin A status estimates
3. New data on the composite coefficient (FaS) to use for calculations in selected population groups
4. New data on simplifications to the field protocol
5. New calculation tool for use by non-modellers
6. A clear field manual with interpretation guidelines for use in population surveys
7. Publications in the form of scientific reports and peer-reviewed papers and conference presentations

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 or to **Dr Pernille Kaestel**. The forms can be downloaded from the **CRA website**.

For more information about research contracts and research agreements, please visit **our web-site**.

Deadline for submission of proposal

Proposals must be received **no later than 1 September 2021**. Transmission via Email is acceptable if all required signatures are scanned.

For additional information, please contact:

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