Vitamin A

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Related nutrients or biomarkers: iodine, iron, zinc

Related measurements: Population coverage of vitamin A interventions (e.g. fortified foods; infant/young child high-dose vitamin A supplementation programme); population prevalence of xerophthalmia, night blindness, measles (infection/mortality)

Importance of vitamin A for global health

Vitamin A is a fat-soluble vitamin that is required for a wide range of physiological processes including vision, immune function, cell differentiation and proliferation, intercellular communication, reproduction, bone formation and growth (1, 2). Vitamin A deficiency (VAD) is associated with an increased risk of mortality from infectious diseases and is a primary cause of childhood morbidity and mortality in low-income countries. VAD causes xerophthalmia (night blindness, Bitot's spots, corneal

ulceration and scarring), and is the leading cause of preventable blindness in children (2, 3). Night blindness has been reported during pregnancy in women in low-income countries, and is likely a consequence of marginal vitamin A status (3). WHO estimates suggest that 19 million pregnant women (15.3%) and 190 million preschool-age children (33%) were affected by VAD between 1995-2005 in countries at risk of VAD (3). More recent data suggest that the prevalence of deficiency among children 6-59 months of age in low and middle-income countries declined from 39% in 1991 to 29% in 2013; the prevalence of deficiency was highest in sub-Saharan Africa (48%) and south Asia (44%) (4). VAD tends to cluster in families and communities; vitamin A status is assumed to be inadequate in areas with documented deficiency in children and pregnant women (2).

Inadequate vitamin A intake remains common in low and middle-income countries, and is associated with poverty, limited infrastructure, food insecurity, and a high incidence of infectious diseases. Populations at risk of inadequate vitamin A intake include young children, pregnant and lactating women in low-resource settings (2).

Vitamin A refers to retinol and its active metabolites, retinal and retinoic acid (5). Vitamin A is found in the diet as preformed retinol esterified to fatty acids. Preformed retinol is found in animal source foods such as liver, fish liver oils, dairy products, egg yolk, and breast milk. Provitamin A (PVA) carotenoids are found primarily in plant source foods such as dark green leafy vegetables (spinach, kale, etc.) and yellow or orange coloured fruits or vegetables (mango, papaya, carrot, pumpkin, orange-fleshed sweet potato), although PVA-carotenoids can also be found in animal source foods (liver, egg yolk) and in breast milk (1, 5).

β-carotene, α-carotene and β-cryptoxanthin are the most common PVA carotenoids in human diets. PVA carotenoids can be bioconverted to retinol in human intestinal cells (5). Theoretically, β-carotene has twice the vitamin A activity of α-carotene and β-cryptoxanthin based on its chemical structure. When enzymatically cleaved in the central position, β-carotene can yield two molecules of retinol whereas α-carotene and β-cryptoxanthin can yield one molecule of retinol (5). Many diet and host-related factors can affect the bioavailability of PVA-carotenoids (6). Dietary fat and the use of food processing methods that disrupt the food matrix can enhance the bioavailability of PVA-carotenoids. The US Institute of Medicine (IOM) and the FAO/WHO have proposed vitamin A equivalence factors for dietary PVA-carotenoids. The US IOM factors are based on the retinol activity equivalent (RAE), where the 1 µg RAE is equivalent to 1 µg retinol, 12 µg β-carotene and 24 µg α-carotene or β-cryptoxanthin (7). The FAO/WHO factors are based on the retinol equivalent (RE), where 1 µg RE is equivalent to 1 µg retinol, 6 µg β-carotene and 12 µg α-carotene or β-cryptoxanthin (8). The values proposed by the US IOM and the FAO/WHO differ because of a difference in interpretation of data on absorption of dietary PVA-carotenoids (1).

Populations in low-income countries tend to rely mainly on PVA-carotenoids from plant source foods to meet their vitamin A needs because these foods tend to be more accessible and affordable than animal source foods (5). Food fortification is used as a strategy for increasing dietary vitamin A intake in many countries (9) (for more information, see https://www.who.int/nutrition/publications/micronutrients/9241594012/en/). Staple foods such as cooking oil, sugar, wheat flour, and margarine, and processed foods such as infant cereals and snack

foods have been fortified with vitamin A. Biofortification is used as a strategy for increasing intake of dietary PVA-carotenoids by breeding varieties of staple crops such as maize, orange-fleshed sweet potato, cassava and banana that contain higher amounts of PVA-carotenoids than traditional varieties (10) (for more information, see https://www.who.int/elena/titles/bbc/biofortification/en/; and https://www.harvestplus.org/what-we-do/crops). Home-fortification is strategy for increasing dietary VA intake by providing households with sachets of micronutrient powders or micronutrient fortified lipid-based nutrient supplements that can be added to foods prepared in the home for young children (11) (see https://www.who.int/elena/titles/review_summaries/mmp-children/en/).

Supplementation is another strategy that is used to improve vitamin A status in young children in populations at risk of deficiency in low and middle-income countries. The WHO has developed a guideline for providing high-dose vitamin A supplements to children 6-59 months of age (12) (see https://www.who.int/nutrition/publications/micronutrients/guidelines/vas_6to59_months/en/).

Are there populations at risk of excessive vitamin A intakes?

Excessive intake of preformed vitamin A from natural food sources is rare, although adverse health effects have been reported in individuals who consumed polar bear liver, which contains very high amounts of preformed vitamin A (13). High intake of PVA-carotenoids does not result in excessive body stores of vitamin A because intestinal bioconversion of PVA-carotenoids to vitamin A is downregulated when vitamin A status is adequate (5). There is concern that excessive vitamin A intake may occur in populations exposed to multiple vitamin A intervention programmes that provide preformed vitamin A, such as food fortification, high-dose supplementation, and home-fortification interventions (14). New biomarkers are needed to assess risk of excessive vitamin A intake and status. Vitamin A intervention programs should be monitored to assess the efficacy and safety of the interventions.

Human biomarkers of vitamin A status

This section is focused on biochemical biomarkers of vitamin A status. Information on dark adaptation testing and night blindness assessment, which can be used to assess VAD in populations is available elsewhere (15, 16) (for more information, see the WHO VMNIS page: http://apps.who.int/iris/bitstream/handle/10665/133705/WHO_NMH_NHD_EPG_14.4_eng.pdf;jsessionid=E94AB50C5B0C9AE6F975F34E78F3DBF1?sequence=1).

Also, ecological and demographic indicators such as socioeconomic status, food insecurity, infectious disease rates, infant mortality rate, access to safe drinking water, etc. can be useful for assessing VAD in populations, (5, 17). Further, coverage rates of vitamin A intervention programmes such as food fortification and high-dose capsule distribution, can indicate uptake or success of interventions, and thereby the ongoing risk of deficiency. Recent reviews have summarized the various methods of human vitamin A status assessment (5, 14).

Serum retinol concentration

The WHO recommends using serum retinol concentration to assess vitamin A status of a population (5, 17).However, serum retinol has limitations as an indicator of VAD because it is homeostatically controlled, and decreases transiently during infection or inflammation. Nevertheless, the distribution of serum retinol concentrations within a population can be useful for assessing VAD (18). It is important to measure biomarkers of inflammation (serum concentrations of C-reactive protein, α_1-acid glycoprotein) to interpret serum retinol data. Regression analysis can be used to adjust serum retinol concentrations in populations with high rates of inflammation (19). Serum retinol cut-off values of <0.7 µmol/L and <0.35 µmol/L are used for moderate and severe deficiency, respectively, in children (20, 21). A serum retinol cutoff of <1.05 µmol/L is commonly used for moderate deficiency in adults (5). Serum retinol binding protein (RBP) concentration may be used as a proxy for serum retinol concentration.

The WHO recommends using at least two biological indicators of vitamin A status for assessing the public health significance of VAD, or one biological indicator in combination with at least four ecological and/or demographic population risk factors for VAD, such as infant mortality rate, under 5 mortality rate, immunization coverage rates, dietary VA intake, etc. (17) (see https://www.who.int/nutrition/publications/micronutrients/vitamin_a_deficiency/WHO_NUT_96.10/en/).

Serum retinol can be measured by high-performance liquid chromatography (HPLC) (22), or by fluorometry using a portable device (see https://www.bioanalyt.com/product/icheck-fluoro/).

Serum retinol and carotenoids can be measured simultaneously by HPLC (see https://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/vitaec_d_met_aecar.pdf)).

Other methods

Relative dose response tests

The modified relative dose response (MRDR) test is used to assess the adequacy of liver vitamin A stores (5). Briefly, a small oral dose of vitamin A_2 (3,4 didehydroretinol) is administered to an individual and a blood sample is obtained 4-7 hours later for measurement of the serum concentrations of vitamin A_2 and vitamin A_1 (retinol) by HPLC (5, 23). The MRDR ratio is calculated as (plasma vitamin A_2 concentration /vitamin A_1 concentration). An MRDR ratio of ≥0.06 is used as the cutoff value to indicate inadequate liver vitamin A stores. The relative dose response (RDR) test is similar, but two blood samples are required, and a test dose of vitamin A_1 (retinol) is used instead of vitamin A_2. Briefly, after obtaining a fasting, baseline blood sample, a small oral dose of vitamin A_1 is administered to an individual, and a second blood sample is obtained 5 hours later. The serum retinol concentration is measured by HPLC in both the baseline and 5-hour blood samples. The relative dose response is calculated as: [(plasma retinol concentration at 5 h – plasma retinol concentration at 0 h)/(plasma retinol concentration at 5 h)] x 100. An RDR of ≥20% is used as the cut-off value to indicate inadequate liver vitamin A stores (17). Note that the MRDR and RDR tests do not provide quantitative estimates of liver vitamin A stores, and only provide information on adequacy/inadequacy of stores.

Retinol binding protein (RBP) (and biomarkers of inflammation)

Serum RBP concentration can be used as a proxy for serum retinol concentration for assessing VAD in a population (5). Serum retinol and serum RBP concentrations are well correlated generally. However, body mass index, kidney function, and perhaps other factors such as age and physiological status, may affect the relationship between serum retinol and serum RBP (5). It may be preferable to measure both serum retinol and RBP concentrations in a subset of a population to determine the relationship between serum retinol and RBP concentrations, and to establish population-specific cut-offs for RBP (24).

Serum RBP can be measured by a sandwich ELISA assay (25) (for more information, see e.g. http://www.nutrisurvey.de/blood_samples/) or a multiplex immunoarray assay (26) (see http://www.quansysbio.com/multiplex/multiplex-assays/human_micronutrient/). Cut-off values for RBP of <14.7 µg/mL (26) or <0.7 µmol/L (25) have been proposed for inadequate vitamin A status, although population specific cut-offs may be preferable, as mentioned above.

The sandwich ELISA and multiplex immunoarray assays that are used for measurement of RBP can be used to measure multiple serum proteins simultaneously, including biomarkers of inflammation (C-reactive protein, α_1-acid glycoprotein) (see links above).

Retinol isotope dilution technique

The retinol isotope dilution (RID) technique is the only assessment method that provides a quantitative estimate of total body stores of vitamin A (14). The RID technique is based on the principle of isotope dilution. Briefly, stable isotope-labelled vitamin A (^{13}C-retinyl acetate or ^{2}H-retinyl acetate) is administered orally, and total body stores of vitamin A are estimated based on the plasma ratio of isotopically-labelled retinol to non-labelled retinol, which is measured at a single time point or multiple time points depending the method used, and an appropriate prediction equation (14, 27). The equations for estimating total body stores of vitamin A are evolving, and simplified equations have been developed recently that are based on compartmental modelling of plasma retinol kinetic data (28, 29). Recent reviews provide detailed information on the equations that have been used to estimate total body vitamin A stores using the RID method (14, 30). Total body stores of vitamin A can be converted to liver vitamin A concentrations using assumptions regarding liver weight as a percentage of body weight, and the percentage of total body vitamin A that is stored in the liver (5). Because the RID method provides a quantitative estimate of liver vitamin A concentration, it could potentially be used to assess vitamin A status across the continuum of deficient to excessive liver vitamin A stores. However, firm cut-off values have not yet been established for deficient or excessive vitamin A status based on estimated liver vitamin A concentrations. However, proposed cut-off values are available elsewhere (5, 27). The current capabilities and limitations of the RID technique are discussed in a recent review (14). Additional details on the application of compartmental modelling of plasma retinol kinetic data to develop equations for estimating total body vitamin A stores can be found in recent publications (28, 29, 31).

Mass spectrometry (LC/MS/MS (32), GC/isotope ratio/MS (33)) is used to measure stable isotope labelled vitamin A in plasma or serum. The mass spectrometry methods require specialised lab equipment and appropriately-trained personnel. Consultation with experts is recommended to discuss issues related to dosing, sensitivity of the analytical method, and timing and number of blood samples to be collected.

Breast milk retinol

Breast milk retinol concentration provides an assessment of both maternal and infant vitamin A status. Breast milk retinol concentration reflects maternal vitamin A intake and status, and provides information on the risk of inadequate vitamin A intake in predominantly or exclusively breastfed infants (34). Vitamin A is present in the fat portion of the milk, and milk fat varies within a feeding episode (lowest in foremilk, highest in hind milk) and throughout the day, therefore milk samples should be collected and processed carefully (34). Two breast milk collection protocols are commonly used to assess the vitamin A concentration (34). Full milk samples are collected at a standardized time of day (usually mid-morning) by using a manual or electric breast pump to express all of the milk from a breast that has not been used to feed the child for at least one hour. Casual milk samples are collected 30 seconds after the child initiates breastfeeding by removing the child from the breast and hand-expressing a small amount of milk (5-10 mL) into a container (35). Milk fat can separate easily from the aqueous portion; therefore, milk samples must be well-mixed prior to aliquotting for measurement of the vitamin A concentration. Full milk samples are mixed thoroughly and a small sample (~3-5 mL) is aliquotted for analysis of the vitamin A content. The remaining milk is returned to the mother to feed to her baby with a spoon. Full milk samples are representative of the milk an infant consumes during a feeding episode. Similarly, casual milk samples are mixed thoroughly before being aliquotted for measurement of the vitamin A concentration (34).

Breast milk vitamin A concentration can be measured by HPLC (36-38), or by fluorometry using a portable device (39) (see https://www.bioanalyt.com/product/icheck-fluoro;). The vitamin A content of full milk samples is expressed as µmol/L and/or as nmol/g milk fat. The vitamin A content of casual milk samples is expressed as nmol/g milk fat because retinol is found in milk fat, and the fat content of casual samples can be highly variable.

Milk fat can be measured by using the crematocrit method (40) . A crematocrit centrifuge is commercially available (EKF Crematrocrit Plus™) (see https://www.fishersci.com/shop/products/separation-technology-creamatocrit-plus-centrifuge/23550151#?keyword=creamatocrit+centrifuge). Cutoff values for low breast milk vitamin A concentrations are <1.05 µmol/L (full milk samples) or <28 nmol/g milk fat (full or casual milk samples).

Serum retinol on dried blood spots

Dried blood spots have been used to measure retinol concentration, although because results are inconsistent possibly due to poor extraction and/or instability of retinol, DBS are not commonly used. However, recent data suggest that retinol (41) and RBP (42) can be measured reliably from DBS in

well-nourished populations. More data are needed to determine whether the DBS methods are reliable in populations with low serum retinol and RBP concentrations. DBS methods are advantageous in field studies because the samples do not require centrifugation or refrigeration, however, more information is needed on stability of DBS in various environmental conditions. Though DBS specimens offer many advantages, samples must be carefully prepared and stored. See https://www.open-global.kcl.ac.uk/common-methods/ for further information.

Serum retinyl esters

The fasting serum retinyl ester concentration can be used to assess excessive vitamin A status (5). Serum retinyl esters increase transiently after consumption of vitamin A, but are low in the fasted state. In contrast, in patients with hypervitaminosis A, fasting retinyl esters are elevated (43). It is essential to obtain fasting blood when using serum retinyl ester concentration as an indicator of excessive vitamin A status. The serum retinyl ester concentration can be measured by HPLC (44). A firm cut-off value for serum retinyl esters has not yet been established. However, a cut-off value of >10% of total serum vitamin A as retinyl esters has been proposed (5). Note that it can be challenging to obtain fasting blood samples from young children in community settings.

Quality Control and technical assistance

Collection and storage of samples

Recommendations on sample collection for serum, plasma, or blood-spot assays are given under "Assay Specific Queries", of the BOND Review on Vitamin A, available here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4997277/ (see page 1839S of the pdf download).

In general blood samples should be protected from light and kept cool but not frozen until they are delivered to the lab for processing, analysis or storage; DBS should be thoroughly dried (in humid climates) before being stored.

Standard reference materials

Standard reference materials are available from the National Institute of Standards and Technology NIST (SRM 1950 Metabolites in Human Plasma).

Confounders of vitamin A deficiency assessment

Zinc or protein deficiency Concurrent zinc and/or protein deficiency can lower hepatic synthesis of retinol binding protein, resulting in lower plasma/serum retinol concentrations (5)

Iron deficiency Concurrent iron deficiency can reduce hepatic mobilization of retinol, resulting in lower plasma/serum retinol concentration (45).

Pregnancy During pregnancy, haemodilution affects serum retinol concentrations, resulting in lower plasma/serum retinol concentrations (5)

Concomitant infections As mentioned above, the acute phase response has a transient negative effect on serum retinol concentrations, thus inflammatory markers (C-reactive protein and α_1-acid glycoprotein) should be measured to interpret serum retinol data. Regression analysis can be used to adjust serum retinol concentrations in populations with high rates of inflammation (19).

Obesity Serum RBP concentration may not reflect vitamin A status in obese individuals (5).

Quality assurance

Accreditation schemes

US CDC Vitamin A assessment quality assurance scheme: VITAL-EQA

The US Centers for Disease Control and Prevention (CDC) Vitamin A Laboratory – External Quality Assurance (VITAL-EQA) program is a standardization program designed to provide laboratories measuring nutritional markers in serum with an independent assessment of their analytical performance. The program assists in monitoring the degree of variability and bias in laboratory assays. Information received from the program can be used to eliminate bias or precision problems in the assay system, and confirm the quality of analysis and increase the confidence level of the lab.

Participation in VITAL-EQA is voluntary and free of charge. Results are not used for accreditation or certification.

Details of the scheme are available at https://www.cdc.gov/labstandards/vitaleqa.html

For other laboratory accreditation and validation schemes, see the OpeN-Global laboratory accreditation page at https://open-global.kcl.ac.uk/accreditation/

Technical assistance

For questions on methods of vitamin A assessment or for technical assistance, please contact the OpeN-Global team at https://open-global.kcl.ac.uk/contact/

Useful links

- Institute of Medicine. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: The National Academies Press. doi: 10.17226/10026. https://www.nap.edu/read/10026/chapter/1

- Biomarkers of Nutrition for Development (BOND) – Vitamin A Review https://academic.oup.com/jn/article/146/9/1816S/4584876

Various pages from the World Health Organization (WHO):

- Micronutrient deficiencies http://www.who.int/nutrition/topics/vad/en/

- Vitamin A deficiency global database
  http://www.who.int/vmnis/database/vitamina/en/
- Vitamin A deficiency global prevalence
  http://www.who.int/nutrition/publications/micronutrients/vitamin_a_deficiency/WHO_NUT_95.3/en/
- Indicators for assessing vitamin A deficiency:
  https://www.who.int/nutrition/publications/micronutrients/vitamin_a_deficiency/WHO_NUT_96.10/en/
- Report on the priorities in the assessment of vitamin A and iron status in populations:
  Tanumihardjo SA. Biomarkers of vitamin A status: what do they mean? In: World Health Organization. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010*. Geneva, World Health Organization, 2012.
  http://www.who.int/nutrition/publications/micronutrients/background_paper2_report_assessment_vitAandIron_status.pdf

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