Original Communication

Usefulness of Vitamin A Isotope Methods for Status Assessment: From Deficiency through Excess

Sherry A. Tanumihardjo

University of Wisconsin-Madison, Madison, WI, USA

Abstract: A variety of methods exist to assess vitamin A status of groups and populations. Vitamin A status is usually defined by the liver retinol concentration. Most indicators of status do not measure or estimate liver stores of retinol. Clinical signs only have utility when liver reserves are almost exhausted, and serum retinol concentrations have utility in the zone of overt deficiency. Dose response tests offer more coverage, but cannot distinguish among liver vitamin A stores in the adequate through toxic range. Different countries continue, or are beginning, to add preformed vitamin A to a variety of staple foods through fortification, and vitamin A supplements are still being distributed in many countries, especially to preschool children. Further, provitamin A biofortified crops are currently being released in several countries. Assessing population vitamin A status in response to these interventions needs to move beyond serum retinol concentrations. Indicators that work in the excessive to toxic range of liver reserves are needed. To date, the only indirect indicator that has been validated in this range of liver reserves in animals and humans is the retinol isotope dilution test using deuterium or ^{13}C, which spans the entire liver reserve continuum from deficiency through excess.

Key words: biofortification, fortification, retinol

Introduction

Vitamin A is an essential nutrient needed in the diet as preformed retinyl esters or provitamin A carotenoids. Its uses by the human body are diverse and include the ability to see, fight off disease, and reproduce. Therefore, the importance of vitamin A for humans calls for public health measures of status among individuals and populations. Traditionally, serum vitamin A (retinol) concentrations have been used and are still being applied in evaluation surveys and intervention studies. However, the utility of serum retinol concentrations as a measure of total body reserves (TBR) of vitamin A was questioned decades ago [1]. A couple of quotations from that paper, which was largely drawn from rat studies stated, "the plasma vitamin A level does not enable any conclusion to be drawn as to the body reserves of vitamin A which are mainly represented by the vitamin A concentration in the liver, for it has been shown that there is a correlation between plasma and liver vitamin A concentrations only in cases of extreme hypo- or hypervitaminosis," and "This example shows that determination of vitamin A plasma concentrations is not a reliable basis for assessment of

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

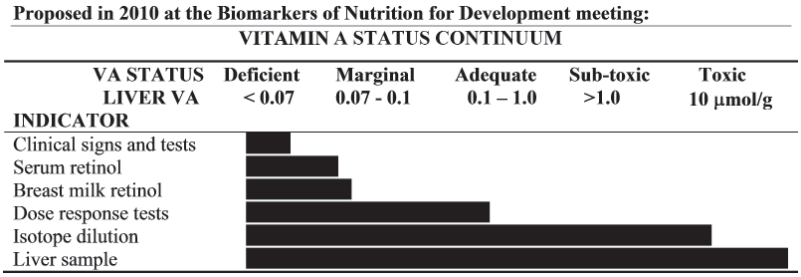

Figure 1: Multiple biomarkers of vitamin A status are used to evaluate interventions and vitamin A status. In reference to a liver sample, which is considered the gold standard, isotope dilution is the only biomarker that has a dynamic range that includes vitamin A deficiency through toxic stores. (Adapted from reference [2]).

the vitamin A status" [1]. This background emphasizes the need for further improving vitamin A assessment methodology that yields more information than serum retinol concentrations for determining vitamin A status of individuals and groups [2].

Methods to assess vitamin A status were recently reviewed [2], and various indicators were aligned with the liver reserve concentration range where they are most useful to predict liver stores of vitamin A if a positive result is found with the test (Figure 1). Clinical signs, such as night blindness and Bitot's spots, only have utility when liver reserves are almost exhausted. Serum retinol concentrations have utility in the zone of overt deficiency but are homeostatically controlled and can "misdiagnosis" groups of individuals. Dose response tests offer a little more coverage, but they also lack utility when vitamin A reserves are above adequate and are not able to distinguish between an adequate, sub-toxic, and toxic vitamin A status. As different countries continue or begin to add preformed vitamin A to a variety of foods in the process of fortification, indicators that work in the excessive to toxic range of liver reserves are needed. To date the only indicator, excluding liver biopsy, that has been validated in this range of liver reserves is the vitamin A-labelled isotope dilution (VALID) test [2].

Serum retinol concentrations

For a variety of reasons, serum retinol concentrations are not an indicator of individual vitamin A status. Retinol circulates in plasma bound to retinol binding protein (RBP) and is a static measure. Further, RBP is a negative acute phase protein and therefore, during infection, RBP will fall and retinol concentrations will be depressed for an undetermined length of time. During times of deficiency, retinol will be recycled more times in an effort to conserve it and decrease catabolism [3]. Plasma retinol concentrations may actually increase first before they drop in response to low liver stores. A recent demonstration of this phenomenon in rats revealed a serum retinol concentration of 1.37 ± 0.21 μmol/L with liver reserves at 0.0051 ± 0.0029 μmol/g liver [4]. Considering that current cutoffs for deficiency are serum retinol concentrations < 0.7 μmol/L and liver reserves of 0.07 μmol/g liver [2], serum retinol did not reflect actual vitamin A status of these rats [4].

Dose response tests

The dose response tests operate on the principle that, as liver reserves of vitamin A become depleted, *apo*-RBP accumulates in the liver because its synthesis does not seem to be regulated. A challenge dose with either retinyl ester for the relative dose response test or 3,4-didehydroretinyl acetate in the modified relative dose response (MRDR) test cause rapid release of the *holo*-RBP complex from the liver due to this accumulation of *apo*-RBP. These tests are appealing because they only require HPLC for analysis; however, during times of excess intakes and high liver reserves of vitamin A, the tests will only indicate adequate status and not excessive. During depleted retinol stores, the tests work well and yield a positive response when liver reserves are <0.1 μmol/g liver, a recently proposed higher liver reserve concentration to indicate deficiency [2]. In areas where vitamin A status is depleted, higher prevalence of positive dose response tests can be used to target vitamin A programs to improve status.

The MRDR test was used to evaluate an intervention study with sweet potato in South African schoolchildren [5]. The MRDR value improved in the group that received the orange sweet potato compared with the white sweet potato ($p = 0.02$), while serum retinol concentrations did not show an intervention effect ($p = 0.15$). Serum retinol actually improved in both treatment groups over time. This may have been due to the deworming of the children or the fact that all children received an extra snack each day as part of the intervention, likely improving overall nutrition.

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

Another recent example of the use of the MRDR test to evaluate an intervention was in Senegalese infants [6]. Serum retinol concentrations only predicted 14.7 % prevalence of vitamin A deficiency, but the MRDR predicted 73.5 % with low liver stores [6]. Thus, although current standards would claim that there is only a mild deficiency, in fact, it is likely that most infants in Senegal do not have enough liver stores of vitamin A to sustain them through the crucial complementary feeding period negatively affecting their immune response to pathogens. Further, the MRDR test was able to detect those infants whose mothers had received high-dose, post-partum supplements ($p = 0.009$). Thus, although the World Health Organization does not recommend post-partum supplements, they can improve the vitamin A status of the nursing infant as predicted and measured in the lactating sow-nursing piglet dyad [7, 8].

Discordant results from serum retinol concentrations and the MRDR test have also been observed in Ghanaian women [9, 10]. Serum retinol concentrations were identical in two geographically distinct groups of Ghanaian women [1.4 and 1.5 μmol/L], but the MRDR test revealed almost a two-fold difference in the serum 3,4-didehydroretinol to retinol ratios between the groups of women with values of 0.048 ± 0.037 and 0.09 ± 0.05. Thus, one group had mostly adequate liver stores and the other did not (abnormal MRDR value is ≥ 0.060). Further, serum retinol concentrations did not respond to their respective interventions but the MRDR test did with significant difference in both a vitamin A supplement [9] and a plant-based intervention [10]. In light of these data and that of many animal studies, the MRDR test appears to give similar answers as serum retinol only when serum retinol is less than about 0.5 μmol/L (i. e., both tests are positive) or greater than about ~1.8 μmol/L (i. e., both tests are negative) [11]. In the middle range of serum retinol concentrations, where most people in the developing world would fall, the MRDR test will better reflect liver reserves of vitamin A and give more indication of status than serum retinol concentrations alone.

Therefore, collectively serum retinol concentrations only reflect liver reserves when they are very low as stated in 1977 [1]. The MRDR test and serum retinol concentrations can give different prevalence rates of vitamin A deficiency as noted in studies in Indonesian children [reviewed in 2], Senegalese infants [6], and Ghanaian women [9, 10]. If true vitamin A status is to be assessed, more sensitive markers of liver reserves need to be used. While the dose response tests have utility in the deficient to adequate range of status, they do not respond when liver reserves become excessive or toxic. Therefore, the main purpose of this paper is to review recent advancements in isotopic methods to evaluate vitamin A status and response to interventions. Dose response tests do not have the same working dynamic range as the VALID methods.

Stable Isotope Methods to Evaluate Vitamin A Stores

Although stable isotope methods to evaluate vitamin A status also date back a couple of decades, the mass spectrometry analysis is not trivial and therefore the methods are not widely utilized without appropriate collaborations [12]. Although the word isotope is often misinterpreted to mean radioactivity, stable isotopes are completely safe to use. Isotopes of any element differ in the number of neutrons that they contain and occur naturally in the environment. The stable isotopes that have been used in vitamin A research include deuterium (^{2}H) and carbon-13 (^{13}C) [12]. The distribution of isotopes in nature is called the natural abundance. For carbon, the natural abundance is 98.9 % ^{12}C, 1.1 % ^{13}C, and only a trace of the radioactive isotope ^{14}C (< 0.00001 %). It is interesting to note that plants have differential uptakes of ^{13}C from the air and some plants will acquire more ^{13}C and are called C4-plants, which are commonly grasses, such as maize and sugarcane. Most leafy green and orange vegetables are C3-plants. This natural shift in ^{13}C-signature was used to evaluate compliance to a weight-loss program based on inclusion of vegetables [13], and was recently applied to evaluate efficacy of orange biofortified maize [unpublished observations]. In both cases the isotope shift worked: less ^{13}C was in the retinol fraction of serum from the vegetable intervention indicating compliance [13], and more ^{13}C was in the serum retinol of people who consumed the orange maize indicating efficacy.

Other uses of stable isotopes in vitamin A research, in addition to assessing vitamin A status, have been to evaluate kinetic behavior and determine bioconversion factors in response to interventions [12]. Several assumptions need to be made when using isotopic methods. For example, one assumes that the heavy isotope behaves like the common isotope, the labelled retinol equilibrates with the total body pool, and the isotope is easily detectable by using small amounts of tracer, which preferably do not perturb the whole body system. Isotope effects caused by differences in mass do occur and the most sensitive methodologies, which use fewer labels on the retinol molecule, will

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

have less isotope effects. Therefore, methods that use the heavier molecules, such as $^{2}H_4$ and $^{2}H_8$ or $^{13}C_{10}$ [reviewed in 12, 14–16], will have more potential mass isotopic effects *in vivo* than a method that uses $^{13}C_2$ [17, 18]. Nonetheless, biological variation in humans is likely more than any analytical discrepancy caused by tracer isotope effects in vitamin A assessment. More noise is actually caused by dose size, which will be covered in the following discussion of mass spectrometers, and assumptions in the calculations.

Mass spectrometers

When determining which isotope method to use, above all else, one needs to know the type of mass spectrometer available in the collaboration and the question being asked. The original isotope dilution test used a typical quadrupole mass spectrometer, which lacked sensitivity and therefore very high doses in the range of 4 to 45 mg were used in children and adults [reviewed in 12]. Application of negative chemical ionization procedures in mass spectrometry allowed smaller doses to be used in humans, and the reported range is 0.5 to 5 mg doses of $^{2}H_8$ and $^{2}H_4$ administered in studies in Chinese and Mexican children [19, 20]. Isotope dilution with ^{13}C-retinol and gas chromatography-combustion-isotope ratio mass spectrometry (GCCIRMS) is the most sensitive methodology available for assessment of vitamin A status. Small doses of lightly enriched $^{13}C_2$-retinol are used, acting like a true tracer [21]. Current recommended doses are 2 μmol (576 μg $^{13}C_2$-retinol) for studies in adults [18] and 1 μmol (288 μg) in children [22]. These doses are less than the current US RDA for vitamin A, *i.e.*, 2.5 and 1.4 μmol vitamin A for women [≥14 years] and children 4 to 8 years old, respectively [23]. With the introduction of multiple vitamin A-fortified foods in the global economy, these doses are still traceable even on the background of excessive stores of vitamin A. In the original work with the ^{13}C-VALID test on monkeys known to have hypervitaminosis A, a 3.5 μmol ^{13}C-retinyl acetate dose was administered, and could still be detected 28 days after dose administration, even though all the monkeys were diagnosed with clinical hypervitaminosis A confirmed by liver biopsy [17]. Thus, this test was validated in a model of toxic stores of vitamin A (i.e., 17.0 ± 6.3 μmol/g liver) caused by highly fortified chow fed every day. This validation is in addition to that with vitamin A-adequate rats that were given three different dose levels of vitamin A daily, where the test had linearity between calculated and measured liver stores of vitamin A [24].

Mass balance equation

Although much more responsive to changes in vitamin A status than serum retinol concentrations, and almost as accurate as a liver biopsy to determine actual liver stores of vitamin A, isotope dilution does require a set of assumptions which work well at the community level, but not necessarily at the individual level. Vitamin A TBR calculations begin using a form of the mass-balance equation [25]:

$(F_a \times a) + (F_b \times b) = (F_c \times c)$

For methods using ^{13}C, "a" is the μmol absorbed from the dose (dose × absorption rate), "b" is TBR in μmol at baseline, and "c" is TBR in μmol after dosing (c = a + b). F_a, F_b, and F_c are the isotope abundance [^{13}C/total C; At%/100; R/(R + 1) and R is ^{13}C/^{12}C] of the dose, baseline serum, and post-dose serum, respectively.

In the application of the ^{13}C-VALID test, 100 % absorption was assumed in well-nourished Americans [18] and hypervitaminotic monkeys [17]. This was based upon the facts that a 1 mg dose, which had radioactive carbon added to it, was almost completely absorbed (99.2 %) in 5 Indian children [26] and no ^{13}C enrichment was detected in the feces of the monkeys. However, in application of the test in groups of individuals who might have micronutrient co-deficiencies and to account for underlying repeated infections, 90 % absorption of the dose is recommended to be used [22]. Further, if study design and resources allow it, researchers or evaluators may want to measure the acute phase protein C-reactive protein at baseline [27]. If elevated >10 mg/L, the child or adult likely had an active infection and the dose may be less well-absorbed, and in this case, 80 % absorption could be assumed [26]. Absorption of a 5 mg dose was 81.2 % in 3 Zambian children without illness using accelerator mass spectrometry for analysis [28]. Smaller doses, such as those recommended for the ^{13}C-VALID test, are likely absorbed more completely, but absorption could be reduced during acute infections. Therefore, researchers should not enroll children or adults into VALID studies who have an active fever at the time of recruitment; should monitor temperature, especially at follow-up time points; and should consider acute phase protein measurements. If widespread use of VALID methods to evaluate vitamin A status of populations occurs, a reduction from 90 to 80 % absorption of the dose overall could be considered if C-reactive protein measurements are not feasible and the groups being studies have a history of repeated infections.

A correction to the equation is commonly used is to account for catabolism of the tracer dose. This correction is made to the TBR calculation:

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

Corrected TBR = $b \times e^{-kt}$, where $k = \ln(2) / (\text{half-life}$ of retinol in days), and t is time in days since dosing.

When the actual half-life of the group being studied is not measured as part of the experimental protocol, the half-life for retinol that has been used is 140 days in adults [18, discussed in 29] and 32 days in young children [22, 30].

Another assumption that needs to be considered is the amount of TBR in the liver to calculate a liver vitamin A concentration. In the original Bausch and Reitz equation, 0.5 was the factor used to correct "a" (the amount of administered dose stored in the liver). This was originally meant to be the amount stored in the liver, which seems to have gotten lost over time and considered to be TBR. The first number obtained in the above equation for the ^{13}C-VALID test is TBR and not liver stores. Therefore, another assumption to estimate the amount in the liver is to use either 50 % storage for groups who have low TBR [22] or 80 % for groups who have adequate TBR [18]. If an actual concentration is desired, this needs to be corrected for liver weight. Traditionally, this percentage is 2.4 % of body weight for adults, 3 % for children, and 4 % for infants [14]. These assumptions and the discrepancy of these percentages are some of the reasons why the VALID test is best applied at the group level and not the individual level.

Methods to Improve Global Vitamin A Status

Vitamin A supplementation programs were implemented in the 1970 s as a short-term action to decrease the prevalence of vitamin A deficiency. The evidence is such that the World Health Organization continues to recommend high-dose supplements to children age 6 through 59 months [31]. However, research evidence does not exist for supplementation in other age groups or in women as a way to decrease infant mortality. In the interim, many countries have started fortifying a variety of foods with preformed vitamin A in an effort to improve the vitamin A status of the whole population [32]. Examples of such programs are sugar fortification in Guatemala [33] and Zambia [34]. However, fortification of products with preformed vitamin A is not without risks. Thus, the new concern is hypervitaminosis with the fortification of multiple staples with preformed vitamin A.

VALID techniques now need to move forward as the method of choice for evaluation of vitamin A fortification programs. In fact, one year after the introduction of vitamin A-fortified sugar in Nicaragua [35], the mean liver reserves of the children enrolled in an evaluation of this program using deuterated retinol increased to 1.2 μmol / g liver (above the sub-toxic cutoff of 1 μmol / g liver, Figure 1). These data were used to model intakes of children in comparison to biofortified foods containing provitamin A carotenoids with predictions of excessive liver reserves over time when using preformed vitamin A as the fortificant [36]. Biofortification is a method of breeding crops with enhanced amounts of nutrients. For vitamin A, this is the process of breeding staple crops like maize or sweet potato [37] or horticultural crops like orange carrots [38, 39] for higher provitamin A carotenoid content.

Biofortification of crops with provitamin A carotenoids is not necessarily a new idea with the domestication of carrot occurring about 1000 years ago and efforts to improve orange color continuing [40], but its application to staple crops has had increasing momentum [37]. The beauty of breeding staple crops with provitamin A carotenoids is that the body will offer a line of protection from hypervitaminosis A due to the regulatory mechanisms in place during the bioconversion of provitamin A carotenoids to retinol [41]. Maize has been biofortified with both β-carotene and β-cryptoxanthin [42, 43]. On a mass basis, these two provitamin A carotenoids have similar bioefficacy [42, 43]. Biofortified maize genotypes have been fed to Zambian children with quick adaptation periods [44, 45]. Further, single test meals of biofortified maize had a favorable bioconversion factor [3.2 ± 1.5 μg β-carotene equivalents to 1 μg of retinol] in Zimbabwean men using the stable isotope reference method [46]. Genotypes of crops, however, can give different bioconversion factors [43]; therefore, crops that are close to commercialization should be tested with VALID methods in the people who will benefit from consumption of biofortified foods.

Study Design and Sample Size Considerations

Originally, the Vitamin A Tracer Task Force suggested sample size estimates of 15 subjects for supplement studies with preformed vitamin A to 30 subjects for intervention studies with provitamin A carotenoids [47] using the paired VALID technique [14]. However, most of the work at the time these bulletins were prepared was performed in people who had vitamin A deficiency and would therefore have a larger response to a trial with provitamin A carotenoids. In consideration

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

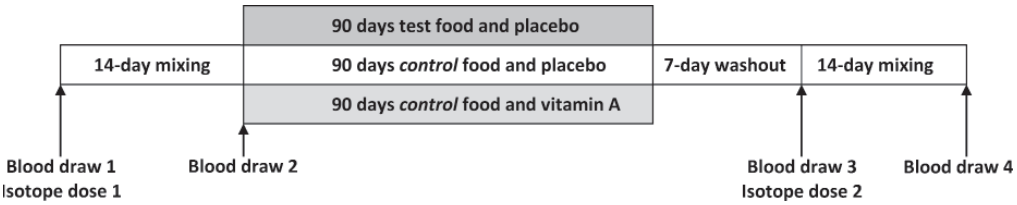

Figure 2: A proposed study design for the paired isotope dilution test to evaluate an intervention with a fortified food, biofortified food with enhanced provitamin A carotenoids, or fruit and/or vegetable intervention. In a highly controlled study, it is recommended that the foods served during the 14-day mixing period be identical. Although a positive control group with preformed vitamin A is recommended when evaluating a provitamin A carotenoid intervention for comparison and calculation of the bioconversion factor, it may not always be necessary for a food fortified with preformed vitamin A. In this figure, *control* food would be a similar food to the test food that is neither fortified nor biofortified.

of sample size, researchers are encouraged to consider anticipated changes in TBR or liver concentrations in vitamin A in response to the intervention as a starting point and add margins of error for variability and analytical losses.

A recent study did not show a difference in TBR using the VALID test in a sweet potato intervention trial in Bangladeshi women for 60 days [48]. Thus, researchers should not only consider larger sample sizes, but also longer periods of time for interventions with provitamin A carotenoids. Figure 2 illustrates a potential study design using the paired VALID test with both positive and negative control groups. After a baseline blood sample, the stable isotope labelled vitamin A is administered, and a mixing time for equilibration of the dose is allowed before the test food is fed. During this mixing period, it is preferable to feed a low vitamin A-containing controlled diet (*control* food in Figure 2). Thereafter, a second fasting blood sample is taken and the intervention can begin. By keeping the diet low in vitamin A, the difference in specific activity between the liver and the serum is kept closer to 1 and therefore not needed in the equation as a correction. This is especially true for the ^{13}C-VALID test because the dose administered is small compared with the deuterated test where a substantial amount of the tracer might be stored in the liver. After a suitable intervention period (90 days in Figure 2), a washout period is recommended so that the final provitamin A carotenoid from the meal and the vitamin A from the positive control group can equilibrate with the total body pool (7 days in Figure 2). After this, another fasting blood sample is taken to determine the residual amount of tracer from the baseline assessment of TBR followed by another isotope dose. After a two-week mixing period, another blood sample is taken to determine the TBR of vitamin A after the intervention. From these data, the change in TBR due to the intervention can be determined by treatment group, and the bioconversion factor can be calculated in reference to the positive and negative control groups.

Variations to the VALID test exist. For example, if $^{2}H_{4}$-retinyl acetate is administered at baseline, sometimes $^{2}H_{8}$ is administered at follow-up. This would allow the researchers the liberty of not having a blood sample at timepoint three [14]. While the initial baseline blood sample is optional for the ^{13}C-VALID test as long as a reference group is used [22], the third blood sample is necessary because of the residual ^{13}C that would be measurable after the intervention by the GCCIRMS. This reiterates the importance of the collaborating group of researchers to understand the subtleties of dose size, blood volume to be collected, and number of blood samples needed for the successful conduct of intervention trials.

Conclusions

The world needs to consider other methods than serum retinol concentrations to assess vitamin A status and evaluate interventions. Considering the addition of preformed vitamin A to a variety of staple foods at the national level in many countries, we now have a new way of looking at optimal stores of vitamin A. People who will be consuming more than one staple that is fortified with preformed vitamin A may now have too high of stores leading to imbalance [49]. Intake of provitamin A carotenoids through biofortification and dietary diversification do not have this same risk [32, 36, 41, 49]. While the ramifications of hypervitaminosis A are not entirely known, bone health is of particular concern [50]. Lifelong exposure to high amounts of preformed vitamin A is now a concern in both developed and developing nations through fortification. To date, the only indirect biomarkers of vitamin A status that are diagnostic across the continuum of TBR are VALID techniques. Improvements in mass spectrometry or analytical simplifications may increase the world-wide application of this sensitive methodology and should be considered.

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

Acknowledgments

The author thanks Bryan Gannon for reviewing the manuscript for content and assistance in preparing Figure 2. Research reviewed in this paper from the author's laboratory was funded by the International Atomic Energy Agency, the National Institutes of Health, United States Department of Agriculture, and HarvestPlus. The views expressed do not necessarily reflect those of the funders.

References

1. Bausch, J. and Rietz, P. (1977) Method for the assessment of vitamin A liver stores. Acta. Vitamin. Enzymol. 31, 99–112.

2. Tanumihardjo, S.A. (2011) Vitamin A: Biomarkers of nutrition for development. Am. J. Clin. Nutr. 94, 658S–665S.

3. Green, M.H. and Green, J.B. (1994) Vitamin A intake and status influence retinol balance, utilization and dynamics in rats. J. Nutr. 124, 2477–2485.

4. Riabroy, N., Dever, J. and Tanumihardjo, S.A. (2014) α-Retinol and 3,4-didehydroretinol support growth in rats when fed at equimolar amounts and α-retinol is not toxic after repeated administration of large doses. Br. J. Nutr. 111, 1373–1381.

5. van Jaarsveld, P.J., Faber, M., Tanumihardjo, S.A., Nestel, P., Lombard, C.J. and Benadé, A.J. (2005) β-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed by the modified-relative-dose-response test. Am. J. Clin. Nutr. 81, 1080–1087.

6. Agne-Djigo, A., Idohou-Dossou, N., Kwadjode, K.M., Tanumihardjo, S.A. and Wade, S. (2012) High prevalence of vitamin A deficiency is detected by the modified relative dose-response test in six-month-old Senegalese breast-fed infants. J. Nutr. 142, 1991–1996.

7. Penniston, K.L., Valentine, A.R. and Tanumihardjo, S.A. (2003) A theoretical increase in infants' hepatic vitamin A is realized using a supplemented lactating sow model. J. Nutr. 133, 1139–1142.

8. Valentine, A.R. and Tanumihardjo, S.A. (2005) One-time vitamin A supplementation of lactating sows enhances hepatic retinol of offspring independent of dose size. Am. J. Clin. Nutr. 81, 427–433.

9. Tchum, S.K., Tanumihardjo, S.A., Newton, S., de Benoist, B., Owusu-Agyei, S., Arthur, F.K.N. and Tetteh, A. (2006) Evaluation of vitamin A supplementation regimens in Ghanaian postpartum mothers using the modified-relative-dose-response test. Am. J. Clin. Nutr. 84, 1344–1349.

10. Tchum, S.K., Newton, S., Tanumihardjo, S.A., Arthur, F.K.N., Tetteh, A. and Owusu-Agyei, S. (2009) Evaluation of a green leafy vegetable intervention in Ghanaian postpartum mothers. Afr. J. Food. Agric. Nutr. Development. 9, 1294–1308.

11. Tanumihardjo S.A. (2012) Biomarkers of vitamin A status: What do they mean? In: WHO. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010.* Geneva, World Health Organization, pp. 44–54.

12. Furr, H.C., Green, M., Haskell, M., Mokhtar, N., Nestel, P., Newton, S., Ribaya-Mercado, J., Tang, G., Tanumihardjo, S.A. and Wasantwisut, E. (2005) Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. Public Health Nutr. 8, 596–607.

13. Howe, J.A., Valentine, A.R., Hull, A.K. and Tanumihardjo, S.A. (2009) ^{13}C Natural abundance in serum retinol acts as a biomarker for increases in dietary provitamin A. Exp. Biol. Med. 234, 140–147.

14. Haskell, M., Ribaya-Mercado, J. and the Vitamin A Tracer Task Force. (2005) Handbook on vitamin A tracer dilution methods to assess status and evaluate intervention programs. *HarvestPlus.*

15. Tang, G. (2012) Techniques for measuring vitamin A activity from β-carotene. Am. J. Clin. Nutr. 96, 1185S–1188S.

16. Haskell, M.J. (2012) The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion – evidence in humans. Am. J. Clin. Nutr. 96, 1193–1203.

17. Escaron, A.L., Green, M.H., Howe, J.A. and Tanumihardjo, S.A. (2009) Mathematical modeling of serum ^{13}C-retinol in captive rhesus monkeys provides new insights on hypervitaminosis A. J. Nutr. 139, 2000–2006.

18. Valentine, A., Davis, C. and Tanumihardjo, S.A. (2013) Vitamin A isotope dilution predicts liver stores in line with long-term vitamin A intake above the current Recommended Dietary Allowance for young adult women. Am. J. Clin. Nutr. 98, 1192–1199.

19. Li, L., Zhao, X., Wang, J., Muzhingi, T., Suter, P.M., Tang, G. and Yin, S. (2012) Spirulina can increase total-body vitamin A stores of Chinese school-age children as determined by a paired isotope dilution technique. J. Nutr. Sci. 1, 1–7.

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

20. Lopez-Teros, V., Quihui-Cota, L., Méndez-Estrada, R., Grijalva-Haro, M.I., Esparza-Romero, J., Valencia, M.E., Green, M.H., Tang, G., Pacheco-Moreno, B.I., Tortoledo-Ortiz, O. and Astiazaran-Garcia, H. (2013) Vitamin A-fortified milk increases total body vitamin A stores in Mexican preschoolers. J. Nutr. 143, 221–226.

21. Tanumihardjo, S.A. (2001) Synthesis of 10, 11, 14, 15-$^{13}C_4$-and 14, 15-$^{13}C_2$-retinyl acetate. J. Label. Compd. Radiopharm. 44, 365–372.

22. Pinkaew, S., Wegmuller, R., Wasantwisut, E., Winichagoon, P., Hurrell, R.F. and Tanumihardjo, S.A. (2014) Triple-fortified rice containing vitamin A reduced marginal vitamin A deficiency and increased vitamin A liver stores in school-aged Thai children. J. Nutr. 144, 519–524.

23. Institute of Medicine. Food and Nutrition Board. (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, pp. 65–126.

24. Tanumihardjo, S.A. (2000) Vitamin A status assessment in rats with $^{13}C_4$-retinyl acetate and gas chromatography/combustion/isotope ratio mass spectrometry. J. Nutr. 130, 2844–2849.

25. Goodman, K.J. and Brenna, J.T. (1992) High sensitivity tracer detection using high-precision gas chromatography-combustion isotope ratio mass spectrometry and highly enriched [U-^{13}C]-labeled precursors. Anal. Chem. 64, 1088–1095.

26. Sivakumar, B. and Reddy, V. (1972) Absorption of labelled vitamin A in children during infection. Br. J. Nutr. 27, 299–304.

27. Clyne, B. and Olshaker, J. (1999) The C-reactive protein. J. Emerg. Med. 17, 1019–1025.

28. Aklamati, E., Mulenga, M., Dueker, S., Buchholz, B.A., Peerson, J.M., Kafwembe, E., Brown, K.H. and Haskell, M.J. (2010) Accelerator mass spectrometry can be used to assess vitamin A metabolism quantitatively in boys in a community setting. J. Nutr. 140, 1588–1594.

29. Olson, J.A. (1987) Recommended dietary intakes (RDI) of vitamin A in humans. Am. J. Clin. Nutr. 45, 704–716.

30. Haskell, M.J., Lembcke, J.L., Salazar, M., Green, M.H., Peerson, J.M. and Brown, K.H. (2003) Population-based plasma kinetics of an oral dose of [$^2H_4$]retinyl acetate among preschool-aged, Peruvian children. Am. J. Clin. Nutr. 77, 681–686.

31. WHO. (2011) *Guideline: Vitamin A supplementation in infants and children 6–59 months of age.* Geneva, World Health Organization.

32. Tanumihardjo, S.A. and Furr, H.C. (2013) International efforts to eradicate vitamin A deficiency. In *Carotenoids and Human Health*; Tanumihardjo, S.A. Ed.; Springer Science and Business Media, New York, NY, pp. 317–324.

33. Dary, O. and Mora, J.O. (2002) Food fortification to reduce vitamin A deficiency: International Vitamin A Consultative Group recommendations. J. Nutr. 132, 2927S–2933S.

34. Clewes, C. and Kankasa, C. (2003) Report of the National Survey to evaluate the impact of vitamin A interventions in Zambia in July and November 2003. National Food and Nutrition Commission of Zambia.

35. Ribaya-Mercado, J.D., Solomons, N.W., Medrano, Y., Bulux, J., Dolnikowski, G.G., Russell, R.M. and Wallace, C.B. (2004) Use of the deuterated-retinol-dilution technique to monitor the vitamin A status of Nicaraguan schoolchildren 1 y after initiation of the Nicaraguan national program of sugar fortification with vitamin A. Am. J. Clin. Nutr. 80, 1291–1298.

36. Tanumihardjo, S.A. (2008) Food-based approaches for ensuring adequate vitamin A nutrition. Compr. Rev. Food Sci. Food Safety. 7, 373–381.

37. Pixley, K., Palacios-Rojas, N., Babu, R., Mutale, R., Surles, R. and Simpungwe, E. (2013) Biofortification of maize with provitamin A carotenoids. In: *Carotenoids and Human Health*; Tanumihardjo, S. A. Ed.; Springer Science and Business Media, New York, NY, pp. 271–292.

38. Simon, P.W., Bowman, M.J. and Tanumihardjo, S.A. (2013) Horticultural crops as a source of carotenoids. In: *Carotenoids and Human Health*; Tanumihardjo, S.A. Ed.; Springer Science and Business Media, New York, NY, pp. 293–301.

39. Mills, J.P., Simon, P.W. and Tanumihardjo, S.A. (2008) Biofortified carrot intake enhances liver antioxidant capacity and vitamin A status in Mongolian gerbils. J. Nutr. 138, 1692–1698.

40. Tanumihardjo, S.A., Bouis, H., Hotz, C., Meenakshi, J.V. and McClafferty, B. (2008) Biofortification of staple crops: An emerging strategy to combat hidden hunger. Compr. Rev. Food Sci. Food Safety. 7, 329–334.

41. Tanumihardjo, S.A., Palacios, N. and Pixley, K.V. (2010) Provitamin A carotenoid bioavailability: What really matters? Int. J. Vitam. Nutr. Res. 80, 336–350.

42. Davis, C., Jing, H., Howe, J.A., Rocheford, T. and Tanumihardjo, S.A. (2008) β-Cryptoxanthin from

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.

supplements or carotenoid-enhanced maize maintains liver vitamin A in Mongolian gerbils [*Meriones unguiculatus*] better than or equal to β-carotene supplements. Br. J. Nutr. 100, 786–793.

43. Schmaelzle, S., Gannon, B., Crawford, S., Arscott, S.A., Goltz, S., Palacios-Rojas, N., Pixley, K.V., Simon, P.W. and Tanumihardjo, S.A. (2014) Maize genotype and food matrix affect the provitamin A carotenoid bioefficacy from staple and carrot-fortified feeds in Mongolian gerbils (*Meriones unguiculatus*). J. Agric. Food Chem. 62, 136–143.

44. Nuss, E.T., Arscott, S.A., Bresnahan, K., Pixley, K.V., Rocheford, T., Hotz, C., Siamusantu, W., Chileshe, J. and Tanumihardjo, S.A. (2012) Comparative intake of white- versus orange-colored maize by Zambian children in the context of promotion of biofortified maize. Food Nutr. Bull. 33, 63–71.

45. Schmaelzle, S., Kaliwile, C., Arscott, S.A., Gannon, B., Masi, C. and Tanumihardjo, S.A. (2014) Nutrient and non-traditional food intakes by Zambian children in a controlled feeding trial. Food Nutr. Bull. 35, 60–67.

46. Muzhingi, T., Gadaga, T.H., Siwela, A.H., Grusak, M.A., Russell, R.M. and Tang, G. (2011) Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men. Am. J. Clin. Nutr. 94, 510–519.

47. Vitamin A Tracer Task Force. (2004) Appropriate uses of vitamin A tracer (stable isotope) methodology. Washington: ILSI.

48. Jamil, K.M., Brown, K.H., Jamil, M., Peerson, J.M., Keenan, A.H., Newman, J.W. and Haskell, M.J. (2012) Daily consumption of orange-fleshed sweet potato for 60 days increased plasma β-carotene concentration but did not increase total body vitamin A pool size in Bangladeshi women. J. Nutr. 142, 1896–902.

49. Tanumihardjo, S.A. (2013) Vitamin A and bone health: The balancing act. J. Clin. Densitom. 16, 414–419.

50. Penniston, K.L. and Tanumihardjo, S.A. (2006) The acute and chronic toxic effects of vitamin A. Am. J. Clin. Nutr. 83, 191–201.

Sherry A. Tanumihardjo

University of Wisconsin-Madison
1415 Linden Drive
Madison, WI 53706
USA
sherry@nutrisci.wisc.edu

Int. J. Vitam. Nutr. Res. 84 (S1) © International Atomic Energy Agency 2014. Published by Hans Huber Publishers, Hogrefe AG, Bern.
All rights reserved. Distributed under the Hogrefe OpenMind license (http://dx.doi.org/10.1027/a000001).
Free access provided with a grant from the International Atomic Energy Agency.