Original Communication

Vitamin A Value of Plant Food Provitamin A – Evaluated by the Stable Isotope Technologies

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Abstract: Humans need vitamin A and obtain essential vitamin A by conversion of plant foods rich in provitamin A and/or absorption of preformed vitamin A from foods of animal origin. The determination of the vitamin A value of plant foods rich in provitamin A is important but has challenges. The aim of this paper is to review the progress over last 80 years following the discovery on the conversion of β-carotene to vitamin A and the various techniques including stable isotope technologies that have been developed to determine vitamin A values of plant provitamin A (mainly β-carotene). These include applications from using radioactive β-carotene and vitamin A, depletion-repletion with vitamin A and β-carotene, and measuring postprandial chylomicron fractions after feeding a β-carotene rich diet, to using stable isotopes as tracers to follow the absorption and conversion of plant food provitamin A carotenoids (mainly β-carotene) in humans. These approaches have greatly promoted our understanding of the absorption and conversion of β-carotene to vitamin A. Stable isotope labeled plant foods are useful for determining the overall bioavailability of provitamin A carotenoids from specific foods. Locally obtained plant foods can provide vitamin A and prevent deficiency of vitamin A, a remaining worldwide concern.

Key words: stable isotopes, vitamin A, provitamin A carotenoids, β-carotene, intrinsic labeling, bio absorption, bio conversion

Vitamin A is an essential nutrient for humans. The human body obtains vitamin A from preformed vitamin A through foods of animal origin or from provitamin A carotenoids through foods of plant origin. Plant provitamin A carotenoids in foods have to be processed, cooked, absorbed and converted to vitamin A in the human body, which are the factors affecting the vitamin A value of the plant provitamin A carotenoids. Therefore, it is of importance to quantitatively determine the conversion efficiency of plant provitamin A carotenoids to vitamin A, specifically the vitamin A value of a plant food. In 1930, Moore discovered that provitamin β-carotene can convert to vitamin A [1]. Since then, the vitamin A value of provitamin A carotenoids has been investigated through many approaches involving various technologies. The aim of this paper is to review the progress over the last 80 years following this discovery of the conversion of β-carotene to vitamin A and the development of various techniques including stable isotope technologies to determine the vitamin A values of plant provitamin A.

In 1980, the Recommended Dietary Allowances [2] stated that "Although the enzymatic conversion of

β-carotene to retinol is theoretically quantitative (i. e., one mole of β-carotene yielding two moles of retinol), because of physiological inefficiency the maximum conversion demonstrable with low doses in animals is about 50 percent on a weight basis. In addition to inefficiency of conversion, there is a widely variable efficiency of intestinal absorption from different food sources. For man, the average absorption is estimated to be one third of the provitamins ingested. (Retinol is assumed to be completely absorbed.) The overall utilization of β-carotene is therefore taken as one-sixth that of retinol. Other carotenoids that have vitamin A activity (e. g., α-carotene, β-cryptoxanthin) are only one half as active as β-carotene; their efficiency as vitamin A sources is taken as one-twelfth that of retinol." Therefore, β-carotene conversion to retinol was estimated as 6 to 1 by weight and for the other provitamin A carotenoids, such as α-carotene, β-cryptoxanthin, etc, conversion to retinol was estimated as 12 to 1 by weight. It is known that both β-carotene and vitamin A can be detected in human blood circulation. In the 1960 s, the estimation of the average absorption of β-carotene for man was based on the studies using radioactive β-carotene [3, 4]. In addition, vitamin A in circulation of well- nourished subjects is homeostatically controlled. Therefore, with well-nourished subjects, depletion-repletion procedures were used to determine the vitamin A value of β-carotene [5, 6].

For a population with compromised vitamin A status (marginal vitamin A deficiency), it is possible to estimate the conversion efficiency of dietary provitamin A carotenoids to vitamin A by measuring changes in serum retinol after dietary intervention [7, 8]. For example, in a study that fed 7–11 year old children for 9 weeks with 500 retinol equivalent (RE) as either orange fruit, green/yellow vegetables, retinol-rich foods, or low vitamin A, low β-carotene foods, it was found that the provitamin A carotenoid conversion efficiency was 12 to 1 by weight in orange fruit, and 26 to 1 in green and yellow vegetables [7]. Another study fed green/yellow vegetables or light colored vegetables [9] for 10 weeks to 5–6 year old children in the fall season in China. In the group fed green / yellow vegetables, a loss of whole body vitamin A reserves was prevented as compared with the group fed light colored vegetables. Based on the analysis of the results from the paired, labeled vitamin A dilution technique, the equivalent of green/yellow vegetable provitamin A carotenoids to vitamin A was about 27 to 1, by weight. A study using the triacylglycerol rich lipoprotein (TRL) response method with the labeled vitamin A as a control to evaluate raw carrot β-carotene conversion to retinol in an adult subject [10] found a conversion efficiency of 13 to 1 by weight. Using the above TRL method and labeled vitamin A as a reference dose, the intake of 10 g of red palm oil by 12 adult subjects showed a conversion efficiency of red palm oil β-carotene to retinol of 5.7 to 1 by weight [11].

Evidently, the conversion efficiency of β-carotene and other provitamin A carotenoids set before 1980 were not 6 to 1 or 12 to 1 by weight as was previously summarized. Based on the data evaluated in 2001 using various dietary plant foods rich in provitamin A carotenoids, the Dietary Reference Intakes for Vitamin A [12] changed the conversion efficiency of provitamin A carotenoids to retinol, characterized as retinol activity equivalents (RAE), to 12 to 1 for β-carotene, and 24 to 1 (by weight) for α-carotene, and β-cryptoxanthin.

The vitamin A conversion of dietary provitamin A carotenoids is not as efficient as had been expected, which can be seen in other studies. In a study of breast feeding women [13] who for 10 weeks consumed 5 mg of β-carotene per day as fruit, leafy vegetables, retinol rich foods, or low vitamin A-low β-carotene foods, the provitamin A carotenoids conversion efficiency in fruit was 12 to 1, and in leafy vegetables was 28 to 1, by weight.

As suggested above, many factors may affect the vitamin A value of plant provitamin A carotenoids in our foods, including processing, cooking, absorption, and eventually metabolic conversion to vitamin A in the human body. To evaluate these factors, the paired vitamin A dilution technique was developed and used to measure changes of body stores of vitamin A after dietary intervention. Haskell et al [14] used the isotope dilution method (10 mg of $^{2}H_{4}$ -retinyl acetate before and after the intervention) with adults (14 / group) fed 750 μg of retinol equivalent per day in either sweet potato or Indian spinach, as compared to groups given a β-carotene capsule or retinyl palmitate for 60 days. They found that the conversion efficiency on a weight basis was about 13 to 1 for sweet potato, about 10 to 1 for Indian spinach, and about 6 to 1 for synthetic β-carotene. The study also suggested that vitamin A status may affect the absorption and bioconversion of β-carotene to vitamin A. In addition, recent reports using a paired isotope method to evaluate the effectiveness of the daily intake of spirulina by Chinese school children showed the improvement of their vitamin A nutritional status [15].

To estimate the efficiency of the absorption and conversion of β-carotene to vitamin A, an isotope reference method has been developed and used to

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evaluate the bioconversion of pure β-carotene [16] and β-carotene in each of spinach, carrots [17], spirulina [18], Golden Rice [19, 20], and yellow maize [21]. The isotope reference method has used intrinsic labeling with the targeted enrichment profile of the provitamin A in the plant food, and synthetic isotope labeled vitamin A as a reference dose. Advanced mass spectrometers are needed for the high sensitivity and analysis on the enrichment of the doses and biological samples at the physiologic level. In general, when studying the vitamin A value of provitamin A carotenoids from plant foods, it is important to intrinsically label the food/dose consumed. The plant food can be grown and harvested by a hydroponic system with about 25 atom % heavy water and plant growing nutrients in the solution [22]. It is preferable to use an isolated system to recycle the heavy water to reduce the loss of the heavy water from evaporation. The harvested plant food must be analyzed for enrichment before using it for the research study. The principles of the study design are shown in Figure 1 and a general procedure for the study is presented in Figure 2. After known amounts of the labeled reference dose (using $^2H_8$ retinyl acetate as an example) and of the plant food intrinsically labeled with $^2H_8$ β-carotene, the analysis of the resulting $^2H_4$ retinol and $^2H_8$ retinol in human circulation enables quantitative determination of the formation of the $^2H_4$ retinol from the $^2H_8$ β-carotene. Thus, this allows an estimation of the conversion efficiency of the $^2H_8$ β-carotene to $^2H_4$ retinol *in vivo*.

Through this approach, conversion factors (all expressed by weight) have been estimated for several plant foods. The conversion of spinach β-carotene to retinol was 21 to 1 in US adults [17] and 7.5 to 1 in Chinese children [18]. The conversion of carrot β-carotene to retinol was 15 to 1 in US adults [17]. The conversion of spirulina β-carotene to retinol was 4.5 to 1 in Chinese adults [18]. The conversion of Golden Rice β-carotene to retinol was 3.8 to 1 in US adults [20] and 2.3 to 1 in Chinese children [19]. The conversion of maize β-carotene was 3.2 to 1 in Zimbabwean adults [21].

These studies not only estimated the efficiency of conversion but also revealed the factors that affect the conversion efficiency. The effect of age and vitamin A nutrition status on the conversion efficiency are shown in the spinach β-carotene studies with conversion of 21 to 1 (adults) vs. 7.5 to 1 (children) as cited in references 17 and 18, and the Golden Rice β-carotene studies with conversion of 3.8 to 1 (adults) vs 2.3 to 1 (children) as cited in references 18 and 20. The studies also confirmed differences related to the food matrixes of plant diets, since the children consuming spinach β-carotene showed a 7.5 to 1 conversion vs. a 2.3 to 1 conversion with Golden Rice β-carotene.

Eighty years after the discovery of β-carotene conversion to vitamin A, efforts to determine the vitamin A value of β-carotene have made steady progress. The techniques using radioactive β-carotene and vitamin A, depletion-repletion with vitamin A and β-carotene, measuring postprandial chylomicron fractions after feeding a β-carotene rich diet, and using stable isotopes as tracers to follow the absorption and conversion of plant food β-carotene in humans have been developed and have increased our understanding of the absorption and conversion of β-carotene to provide vitamin A.

β-Carotene is converted enzymatically into vitamin A, not only in the small intestine, but also by other tissues that convert the absorbed β-carotene to vitamin A in humans. The conversion of β-carotene to vitamin A is dose [23] & age dependent [17, 18, 21]. The efficacy of vitamin A conversion from plant foods rich in β-carotene—natural (spinach, carrots, spirulina), hybridized (high β-carotene yellow maize, sweet potato, cassava, and banana), and bioengineered (Golden Rice)—have shown promising results. The use of these foods, such as spirulina [15], in community settings has shown positive outcomes on body retinol and further such evaluations are needed to demonstrate the effects of various locally available provitamin A rich plant foods on body vitamin A status.

The results from using various techniques, specifically using stable isotope labeled plant foods, will be of practical importance for planning the use of various sources of β-carotene, including local plant foods rich in β-carotene or provitamin A carotenoids, to provide vitamin A and to prevent deficiency of vitamin A in humans.

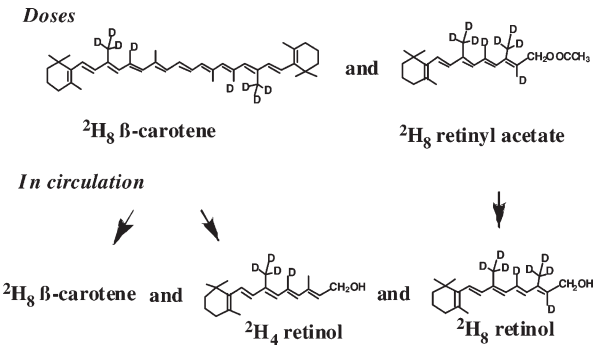

Figure 1: Conversion of labeled $^2H_8$ ß-carotene and $^2H_8$ retinyl acetate into $^2H_4$ retinol and $^2H_8$ retinol, respectively. Here, D = ^2H.

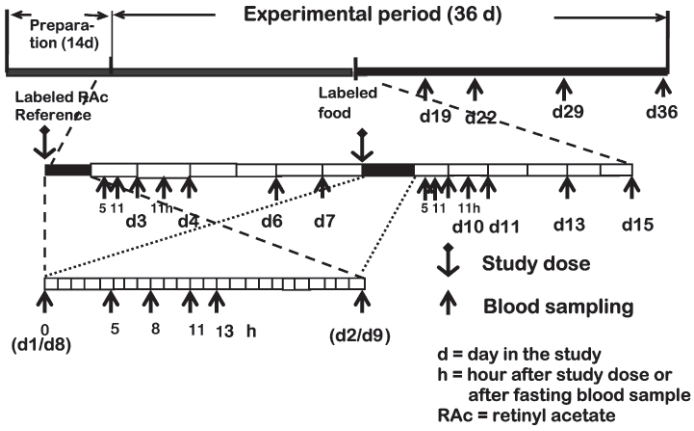

Figure 2: Study procedures of a representative isotope reference method. After 14 day of study preparation period, study subject will contribute a fasting blood sample and then take a labeled retinyl acetate dose. Blood samples will be taken at 5, 8, 11, and 13 h after the retinyl acetate dose. Further, fasting blood samples will be taken at d2 (non-fasting at 5 and 11 h after the fasting blood drawn), d3 (non-fasting at 11 h after the fasting blood drawn), d4, d6, d7. At d7 after the fasting blood sample, a labeled food will be taken by the volunteer and blood samples will be taken at 5, 8, 11, and 13 h after and d9 (non-fasting at 5 and 11 h after the fasting blood drawn), d10 (non-fasting at 11 h after the fasting blood drawn), d11, d13, d15, d19, d22, d29, and d36. Here, d = day.

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References

1. Moore T. (1930) Vitamin A and carotene. Biochem. J. 3, 692.
2. The National Research Council, Institute of Medicine. (1980) Recommended Dietary Allowances, Ninth edition.
3. Goodman, D.S., Blomstrand, R., Werner, B., Huang, H.S., Shiratori, T., (1966) The intestinal absorption and metabolism of vitamin A and β-carotene in man. J. Clin. Invest. 45, 1615.
4. Blomstrand, R. and Werner, B. (1967) Studies on the intestinal absorption of radioactive beta-carotene and vitamin A in man. Conversion of beta-carotene into vitamin A. Scand. J. Clin.Lab. Invest. 19(4), 339.
5. Hume, E.M. and Krebs, A.A. (1949) Vitamin A requirement of adult humans: an experimental study of vitamin A deprivation in man. A report of the vitamin A subcommittee of the accessory food factors committee. Spec. Rep. Ser. Med. Res. Coun. No. 264.
6. Sauberlich, H.E., Hodges, R.E., Wallace, D.L., Kolder, H., Canham, J.E., Hood, J., Raica, N., Jr. & Lowry, L.K. (1974) Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam. Horm. 32, 251.
7. de Pee, S., West, C.E., Permaesih, D., Martuti, S., Muhilal, and Hautvast, J.G.A.J. (1998) Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. Am. J. Clin. Nutr. 68, 1058.
8. Khan, N.C., West, C.E., de Pee, S., Bosch, D., Phuong, H.D., Hulshof, P.J.M., Khoi, H.H., Verhoef, H.and Hautvast, J.G.A.J. (2007) The contribution of plant foods to the vitamin A supply of lactating women in Vietnam: a randomized controlled trial. Am. J. Clin. Nutr. 85, 1112.
9. Tang, G., Gu, X-f., Hu, S-m., Xu, Q-m., Qin, J., Dolnikowski, G.G., Fjeld, C.R., Gao, X., Russell, R.M. and Yin, S-a. (1999) Green and yellow vegetables can maintain body stores of vitamin A in Chinese children. Am. J. Clin. Nutr. 70, 1069.
10. Parker, R.S., Swanson, J.E., You, C-S., Edwards, A.J. and Huang, T. (1999) Bioavailability of carotenoids in human subjects. Proc. Nutr. Soc. 58, 155.
11. You, C-S., Parker, R.S. and Swanson, J.E. (2002) Bioavailability and vitamin A value of carotenes from red palm oil assessed by an extrinsic isotope reference method. Asia Pacific J Clin Nutr. 11(Suppl), S438.
12. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Food and Nutrition Board, Institute of

Medicine, National Academy Press, Washington, DC, 2001 National Academy of Sciences. Institute of Medicine. Food and Nutrition Board.

13. Khan, N.C., West, C.E., de Pee, S., Bosch, D., Phuong, H.D., Hulshof, P.J. M., Khoi, H.H., Verhoef, H., and Hautvast, J.GAJ. (2007) The contribution of plant foods to the vitamin A supply of lactating women in Vietnam: a randomized controlled trial. Am. J. Clin. Nutr. 85, 1112.

14. Haskell, M.J., Jamil, K.M., Hassan, F., Peerson, J.M., Hossan, M.I., Fuchs, G.J. and Brown K.H. (2004) Daily consumption of Indian spinach (Basella alba) or sweet potatoes has a positive effect on total-body vitamin A stores in Bangladeshi men. Am. J. Clin. Nutr. 80, 705.

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

16. Tang, G., Qin, J., Dolnikowski, G.G. and Russell, R.M. (2003) Short-term (intestinal) and long-term (postintestinal) conversion of β-carotene to retinol in adults as assessed by a stable-isotope reference method. Am. J. Clin. Nutr. 78, 259.

17. Tang, G., Qin, J., Dolnikowski, G.G., Russell, R.M., Grusak, M.G. (2005) Spinach or carrot can supply significant amounts of vitamin A as assessed by feeding with intrinsically deuterium-labeled vegetables. Am. J. Clin. Nutr. 82, 821.

18. Wang, J., Wang, Y., Wang, Z., Li, L., Qin, J., Lai, W., Fu, Y., Russell, R.M., Grusak, M.A., Tang, G. and Yin, S. (2008) Vitamin A equivalent of spirulina β-carotene in Chinese adults assessed by stable isotope dilution and reference techniques. Am. J. Clin. Nutr. 87, 1730.

19. Tang, G., Hu, Y., Yin, S., Wang, Y., Dallal, G.E., Grusak, M.A. and Russell, R.M. (2012) Golden Rice β-carotene is as good as β-carotene in oil in providing vitamin A to children. Am. J. Clin. Nutr. 96, 658.

20. Tang, G., Qin, J., Dolnikowski, G.G., Russell, R.M. and Grusak, M.A. (2009) Golden Rice is an effective source of vitamin A. Am. J. Clin. Nutr. 89, 1.

21. Muzhingi, T., Gadaga, H.T., Siwela, A., 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.

22. Grusak, M.A. (1997) Intrinsic stable isotope labeling of plants for nutritional investigations in humans. J. Nutr. Biochem. 8, 164.

23. Tang, G. Techniques for measuring vitamin A activity from β-carotene. Am. J. Clin. Nutr. 96 (suppl), 1185 s.

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