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EVALUATION OF A GREEN LEAFY VEGETABLE INTERVENTION IN GHANAIAN POSTPARTUM MOTHERS

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ABSTRACT

Vitamin A deficiency is a major public health problem in many developing countries and women of childbearing age and children are most affected. Low dietary intakes and increased requirements during different life stages contribute to inadequate vitamin A liver reserves and hence the importance of assessing vitamin A status to determine population groups at risk of deficiency. This study was a community-based, exploratory intervention trial of postpartum mothers selected from villages in Ejisu/Juabeng District of the Ashanti Region in Ghana. The objective was to determine the improvement in vitamin A status of postpartum Ghanaian mothers fed daily with African eggplant leaves using the modified-relative-dose-response (MRDR) test for indirect assessment of liver vitamin A stores. Potential mothers were visited at home by trained field workers 7-10 days after their delivery and recruited into the trial. Baseline MRDR tests were performed for all the women (n = 61). The women were randomly assigned into control (n =10) and intervention (n =51) groups and invited back each month for follow-up MRDR assessment over period of three months. A daily portion of 200 g (2.6 mg of Beta-carotene) of African eggplant leaves were given to mothers in the intervention groups for three months while those in the control group did not receive any additional vegetables. The baseline mean serum retinol concentration and MRDR value were 1.5 ± 0.6 µmol/l and 0.09 ± 0.05 respectively indicating a marginal vitamin A status. Serum retinol concentrations did not differ between (p = 0.47) and within the two groups by time point (p= 0.41). The African eggplant leaves were well accepted and there was a significant improvement in the vitamin A status as assessed by MRDR test within the intervention group over the three months period (P = 0.0001). Vitamin A status also improved in the control group but there was loss of statistical power due to number of dropouts. Dietary modification and nutrition education to women of childbearing age to include natural food sources rich in provitamin A may provide the long-term solution to prevent vitamin A deficiency in developing countries because indigenous leafy vegetables can be easily cultivated in our communities making them sustainable and cheaper compared to periodic oral dosing with vitamin A.

Key words: Vitamin A, 3, 4-Didehydroretinol, -Carotene, Vitamin A status

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INTRODUCTION

The vitamin A status of under-privileged women and children is compromised and this is exacerbated by low socio-economic status [1-4]. Low dietary intakes and increased requirements during different life stages contribute to inadequate vitamin A liver reserves. Assessing vitamin A status is important in determining which population groups are at risk of deficiency [5]. Serum retinol concentrations are homeostatically controlled and do not begin to decline until liver reserves are dangerously low [6]. In times of infection, serum retinol concentrations are reduced due to the acute phase response [7]. Moreover, during acute infection, retinol is excreted in significant amounts in the urine, increasing requirements [8].

Liver reserves less than 0.070 mol retinol/g are considered deficient [9]. The modified relative dose response (MRDR) test is an indirect measure of liver vitamin A reserves. As liver vitamin A reserves become depleted, *apo*-retinol binding protein (RBP) accumulates in the liver. When an oral dose of vitamin A is administered, the freshly absorbed retinol is rapidly liganded with the accumulated *apo*-RBP and is released into plasma; if 3, 4-didehydroretinol ester is given orally, 3, 4-didehydroretinol is also released as its complex with RBP. For the MRDR test, a precise dose of 3, 4-didehydroretinyl acetate is administered and the ratio of 3, 4-didehydroretinol to retinol (MRDR value) in serum at 4 to 6 hours after dosing is determined. Less adequate liver vitamin A stores will result in a greater ratio of 3, 4-didehydroretinol to retinol in serum [5]. The MRDR test distinguishes between moderately inadequate and adequate vitamin A status; MRDR values $\leq$ 0.030 have been defined as indicating vitamin A adequacy in humans, 0.030 to 0.060 is uncertain vitamin A status, and MRDR values more than 0.060 indicate vitamin A deficiency [10].

This study followed a high-dose vitamin A supplementation trial among lactating women in Ghana. Lactating women in Ghana have a marginal vitamin A status, as assessed by the MRDR test, and high-dose supplements were able to protect the women from vitamin A depletion for at least 5 months postpartum [11]. In this study because the vitamin A status of the mothers were defined as marginal, indigenous green leafy vegetables rich in provitamin A may improve their vitamin A status without the high-cost program implications of administering vitamin A capsules.

Multiple factors affect the bioavailability of provitamin A carotenoids from vegetables [12-14]. The MRDR test has successfully been used to monitor change in vitamin A status after sweet potato feeding in South African children [15]. The leaf chosen for this study was from the African eggplant (*Solanum macrocarpon*), which is locally called Gboma and belongs to the S*olanaceae* family. This is a regionally

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consumed, indigenous leafy vegetable (ILV) [16]. Limited research indicates that these vegetables have good nutritive value as they contain high levels of calcium, iron, phosphorus, vitamins and protein [17-21].

Women of reproductive-age living with vitamin A deficiency (VAD) frequently report night blindness during pregnancy and/or lactation [22]. Night blindness in women during periods of increased physiologic need is thought to reflect VAD. In a randomised community trial in pregnant Nepalese women, ocular defects were reduced in their newborns by weekly low-dose vitamin A supplementation [23]. During VAD there is a reduction in the amount of vitamin A needed to support maternal reproductive processes, including foetal growth and development, and to replace vitamin A losses in breast milk during lactation. Many of the epithelial tissues are important barriers to infection and VAD impairs this function [24]. In VAD, keratin-producing cells replace mucus-secreting cells in many epithelial tissues of the body which is the basis of the pathological process known as xerosis in which there is drying of the conjunctiva and cornea of the eye [25].

Studies in pregnant women from Nepal, Philippines, Zambia, Ghana, and Mali have reported prevalence values for subclinical VAD (serum retinol <0.7 mol/L) between 19-38% [11, 26-27]. The objective of this study was to assess the vitamin A status of lactating women given green leafy vegetables by applying the MRDR test over time.

SUBJECTS AND METHODS

Subjects

The study was a community-based, exploratory intervention trial of 77 postpartum mothers selected from villages in Ejisu/Juabeng District of the Ashanti Region in Ghana. The Institutional Ethics Committee (IEC) of the Kintampo Health Research Centre, Ghana Health Service approved the study protocol. Subjects were free to decline to participate in the study and confidentiality of the information collected was assured. To be eligible for recruitment into the trial, mothers were expected to be resident in the villages in Ejisu/Juabeng District and express a willingness to participate in the study by signing a consent form. Eligible mothers had to generally be in good health as determined by a medical history and physical examination.

After baseline MRDR tests were done, 16 mothers refused blood draw and the rest were randomly assigned to two groups. 61 identical small white plastic discs comprising 51 intervention group discs and 10 labeled control group discs were placed in an opaque plastic bag. 61 post partum mothers were assembled at baseline and asked to draw one labeled disc from the bag and depending on which disc was drawn they were allocated to the group indicated on the disc.

Each mother in the intervention group (n = 51) received 200 g (2.6 mg -carotene) of fresh African eggplant leaves daily for three months [28]. Fresh leaves of African

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eggplant *(Solanum macrocarpon)* were collected from the Amanfro Prison Camp farms in Kumasi and transported every three days to the study site for distribution. Mothers in the control group (n = 10) did not receive African eggplant leaves. All women received nutrition education messages concerning the inclusion of vegetables in the diet. Subjects were visited weekly by trained fieldworkers (FWs) and supplied with packets of indigenous leafy vegetables. The FWs checked for compliance, answered questions, and educated the subjects on the importance of vitamin A to the health of mothers and indirectly to their breastfed infant. During monthly blood draws, subjects were further educated on the importance of vitamin A and the coordinator answered questions which the FWs were not able to address. There was a misconception that indigenous leafy vegetables were foods meant for the poor and it was important that these misconceptions were addressed. During the follow-up months, the MRDR test was performed in all women who consented.

Modified relative dose response test

The MRDR test involves giving an oral dose of 8.8 μmol 3, 4-didehydroretinyl acetate dissolved in corn oil in the morning. This amount corresponds to approximately 2500 μg Retinol Equivalents (7500 IU) vitamin A, which is an amount commonly found in prenatal vitamins. However, the biological activity of 3, 4-didehydroretinol may be less than that of retinol [29]. The mothers were dosed at their homes and five hours later a single finger prick blood sample (500 μl) was taken. The blood samples were stored on ice in a light-protected cooler until transported to the laboratory. Clotted blood samples were centrifuged at 600 X g for 10 min, and serum was stored at $-20^{o}C$ until shipped frozen to the University of Wisconsin-Madison (USA) at which time they were stored at $-80^{o}C$ until analyzed.

Extraction and high-pressure liquid chromatography (HPLC) assay

Serum was thawed; 200 μl aliquots were treated with 250 μl ethanol and then extracted thrice with 300 μl hexane. All extractions were done under gold fluorescent lights. Retinyl acetate dissolved in ethanol was used as an internal standard to determine extraction efficiencies. The hexane layers were pooled and the solvent evaporated under argon. The residue was re-dissolved in 40 μl methanol:ethylene dichloride (3:1, v:v), of which 35 μl was injected onto a 5 μm-Waters 'Sunfire' 15 cm reversed-phase column (Milford, Massachusetts, USA). The wavelength of detection was set at 350 nm to optimize 3, 4-didehydroretinol detection and quantification. The mobile phase was 74:20:6 methanol: acetonitrile:water (0.05% triethylamine) at a flow of 1 ml/min. External standards of 3, 4-didehydroretinol and retinol were purified by HPLC and used to quantify the 3, 4-didehydroretinol and retinol in the serum samples. The leaf sample analysis was carried out using HPLC at the Noguchi Memorial Institute for Medical Research, Ghana.

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Statistical analysis

SAS statistical computer software (version 8.2; SAS Institute, Cary, NC, 2001) was used in the analysis of data. A repeated measures analysis of variance table was generated using SAS PROC MIXED, which allows the treatment variances to be different between the groups. The variance ratio (F) was calculated for serum MRDR values and retinol concentrations in relation to subjects' treatment groups and time. The serum MRDR test and retinol concentration were treated as continuous variables. The differences within the treatment groups by time were assessed using the differences of Least Square Means (LSM). Values are presented as means $\pm$ standard deviations; $p < 0.05$ was considered significant.

RESULTS

Socioeconomic factors

The number of women who consented for participation in the study was originally 77. The number of women that agreed to baseline and at least one MRDR follow-up assessment was 61. Thirty-six percent of subjects were between 20 to 29 y, 18% were between 30 to 45 y, and 33% did not know their age. The mean baseline retinol concentration and MRDR values were 1.5 ± 0.6 µmol/l and 0.09 ± 0.05, respectively. Sixty-nine percent of the subjects were married; 75% had attended middle or junior secondary school; and 1% had attended post-middle school, which was the highest level of education.

Vitamin A status

At baseline measurements, 47% and 70% of the women enrolled had insufficient vitamin A liver reserves in the intervention and control groups, respectively (Table 1). Vitamin A insufficiency is defined as a MRDR value ≥ 0.060 and a public health problem exists when > 20% of a community has values ≥ 0.060 [10]. These percentages continued to decrease during the study. The MRDR values indicated an improvement in vitamin A liver reserves from baseline to each of the three follow-up times for both groups (Table 2). The overall effect of time of treatment was significant ($p < 0.0001$). Vitamin A status improved within the intervention group during the study. In the intervention group, follow-up months one and two did not differ from baseline ($p = 0.25$), but all other comparisons differed ($p < 0.05$) using differences of LSM. The control group also had a significant improvement in the MRDR values and did not differ from the intervention group during the study (Table 2), but a large number of dropouts coupled with the smaller initial number enrolled in the control group decreased the confidence in the data to conclude that nutrition education alone can make an impact. Serum retinol concentrations did not differ by group ($p = 0.47$) or time ($p = 0.66$).

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DISCUSSION

The Ghanaian postpartum mothers had marginal liver reserves of vitamin A at baseline as assessed by the MRDR test, which is indirectly related to liver reserves. The mean serum retinol concentrations were high in these women (1.5 ± 0.6 μmol/l) and much higher than what are considered deficient (*i.e.*, < 0.7 μmol/l) or marginal in women (*i.e.*, between 0.7 and 1.05 μmol/l). A previous study of rural Ghanaian women had similar serum retinol concentrations (1.4 ± 0.5 μmol/l), but their mean MRDR value was half that found in this study (0.048 ± 0.037) [11]. These findings contradict a simulated analysis in which the difference found in MRDR values was said to occur because of underlying different serum retinol concentrations [30]. Furthermore, the serum retinol concentrations did not change either within or between groups making it an insensitive indicator of changes in vitamin A status in these Ghanaian women.

The intervention group showed a steady decrease in their MRDR values over the three-month period, indicating an improvement in vitamin A liver reserves. This finding is very similar to a β-carotene supplementation trial in lactating Bangladeshi women where MRDR values decreased with time of supplementation [31]. Another green leafy vegetable feeding study in Indonesian lactating women did not show improvement in liver reserves [32]. Two major differences between the Indonesian study and this study in Ghanaian postpartum women were that the Indonesian women had poorer vitamin A status at baseline (0.81 ± 0.04 to 0.89 ± 0.04 μmol/l) and they were fed less vegetable per day, for example 100-150 g versus 200 g in this study. Therefore, an overall change to adequate vitamin A status may not be expected, especially when the intervention group is considered vitamin A deficient and lactating [26, 33, 34].

The vitamin A status of the Ghanaian women in the control group also showed an improvement during the trial, but the number of women remaining in the final assessment (n = 5) was not statistically powered to conclude that nutrition education alone could make a difference at the community level. Because the nature of the intervention was not blinded, the women could have modified their diets during this project. During this trial the community became aware of the importance of vitamin A on maternal health and this may have influenced women to start eating other indigenous leafy vegetables which were also rich in provitamin A apart from the African eggplant leaves. Although attrition occurred in both groups, the vegetable group maintained enough women to evaluate the vitamin A status using the MRDR test in a longitudinal study. Attrition was due in part to the repeated blood drawing nature of the study in this culture.

The serum retinol concentrations of lactating women in Ghana indicate that they do not have clinical VAD. However, their MRDR values indicate that they have a depleted vitamin A status and will benefit from either supplementation or dietary strategies [11]. Typically, vitamin A supplementation programs are expensive to implement and sometimes coverage rates are not ideal. The intervention study with African eggplant leaves shows promise as a means to improve vitamin A status of postpartum mothers and the community in general. Using the data obtained from the results presented herein, a more carefully controlled study should be designed in separate villages of similar socioeconomic status to see if this rich plant source could improve vitamin A status at the community level in an intervention group versus a community with no intervention.

Although the study was not designed to look at effects of African eggplant leaves on breast milk flow, an interesting observation was made that more than 80% of the postpartum mothers in the intervention group reported an increase in the secretion and flow of breast milk (galactogenic effect) throughout the feeding period while none of the mothers in the control group reported such an observation. This point warrants further evaluation in a controlled trial.

Periodic oral dosing and food fortification have had documented success and have established their merit in preventing nutritional blindness, but they are considered expensive, temporary solutions. Long-term intervention through dietary modification of the intake of foods rich in provitamin A and vitamin A may be more effective [35, 36, 37]. The conversion factor for β-carotene to retinol for green leafy vegetables has been suggested to be 26 μg β-carotene to 1 μg retinol using changes in serum retinol concentrations [38]. However, data using more sensitive stable isotope methods to calculate changes in total body reserves of vitamin A after feeding pureed spinach estimated a conversion factor of 10:1 [39]. Data in a vitamin A-depleted animal model which has predicted that vitamin A status is a major determinant of the conversion factor calculated 3:1 for spinach, brussels sprouts, and kale [40]. Because the women in this study were vitamin A-depleted as assessed by the MRDR test, conversion to retinol was probably favoured after absorption resulting in a high conversion rate.

Biofortification of staple crops with provitamin A carotenoids, *e.g.*, maize and cassava, or switching to more yellow- or orange-coloured local varieties will also enhance vitamin A status [40, 41]. Among the benefits of promoting such natural food sources is that they provide concurrent intake of other nutritive and non-nutritive substances that contribute to the prevention of disease. For example, fruits and vegetables consistently are shown to provide protection against certain types of cancer [42]. Another benefit is the avoidance of the potential toxicity associated with the

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over consumption of vitamin A supplements [40, 43]. Thorough cost-benefit analyses of the three strategies (vitamin A supplementation, food fortification, and dietary interventions) to prevent and control VAD have yet to be made in Ghana. Because of the positive outcomes of this community-based intervention, further work is warranted with a larger sample size to better reflect community impact.

CONCLUSION

Nutrition education and encouraging women to include locally grown green leafy vegetables in their diets could be an important strategy to improve vitamin A status. There is the need to identify their nutritional value due to their potential to overcome micronutrient deficiencies. Dietary modification to include natural food sources rich in provitamin A may provide the long-term solution to prevent VAD in developing countries because indigenous leafy vegetables can be easily cultivated in our communities making them sustainable and cheaper compared to periodic oral dosing with vitamin A.

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Table 1: Percentage of Ghanaian lactating women with insufficient vitamin A liver reserves in the intervention and control groups. Insufficient vitamin A liver reserves were defined as a modified relative dose response (MRDR) value of $\geq$ 0.060.

Time points	Intervention			Control		
	Subjects (n)	Subjects with MRDR>0.060	%	Subjects (n)	Subjects with MRDR>0.060	%
Baseline	51	24	47	10	7	70
Month 1	43	14	33	8	4	50
Month 2	41	11	27	5	1	20
Month 3	34	1	3	5	0	0

Table 2: Effect of feeding with indigenous leafy vegetables on modified relative dose response (MRDR) values over time for lactating Ghanaian women in both intervention and control groups[1]

Time	Green Leaf group MRDR value	Control group MRDR value
Baseline	0.083 ± 0.045^a	0.11 ± 0.060^a
Month 1	0.058 ± 0.027^b	0.068 ± 0.020^b
Month 2	0.049 ± 0.029^b	0.073 ± 0.080^b
Month 3	0.025 ± 0.016^c	0.014 ± 0.010^c

[1]Superscript letters represent differences within each treatment group using differences of least squares means. n = 51, 43, 41 and 34 for the intervention group and n = 10, 8, 5, and 5 for the control group.

REFERENCES

1. **Tanumihardjo SA, Permaesih D, Dahro AM, Rustan E, Muhilal, Karyadi D and JA Olson** Comparison of vitamin A status assessment techniques in children from two Indonesian villages. *Am. J. Clin. Nutr.* 1994; **60:** 136-141.

2. **Tanumihardjo SA, Muherdiyantiningsih, Permaesih D, Dahro AM, Muhilal, Karyadi D and JA Olson** Assessment of the vitamin A status in lactating and nonlactating, nonpregnant Indonesian women by use of the modified-relative-dose- response (MRDR) test. *Am. J. Clin. Nutr.*1994; **60:** 142-147.

3. **Tanumihardjo SA, Suharno D, Permaesih D, Muherdiyantiningsih, Dahro AM, Muhilal, Karyadi D and JA Olson** Application of the modified relative dose response test to pregnant Indonesian women for assessing vitamin A status. *Eur. J. Clin. Nutr.* 1995; **49:** 897-903.

4. **Duitsman PK, Cook LR, Tanumihardjo S and JA Olson** Vitamin A inadequacy in socioeconomically disadvantaged pregnant Iowan women as assessed by the modified relative dose response (MRDR) test. *Nutr. Res.* 1995; **15:** 1263-1267.

5. **Tanumihardjo SA** Assessing vitamin A status: past, present and future. *J. Nutr.* 2004; **134:** 290S-293S.

6. **Underwood BA** Vitamin A in animal and human nutrition. **In:** Sporn MB, Roberts AB, and DS Goodman (Eds). The Retinoids vol. 1. Orlando, FL: Academic Press, 1984: 281-391.

7. **Clausen SW and AB McCoord** The carotenoids and vitamin A of the blood. *J.Pediatr.* 1938; **13:** 635-650.

8. **Stephensen CB, Alvarez JO, Kohatsu J, Hardmeier R, Kennedy JI Jr and RB Gammon Jr.** Vitamin A is excreted in the urine during acute infection. *Am. J. Clin. Nutr.* 1994; **60:** 388-392.

9. **Olson JA** Vitamin A. **In:** Rucker RB, Suttie JW, McCormick DB and LJ Machlin (Eds). Handbook of Vitamins, 3rd edition. New York, NY: Marcel Dekker, 2001: 1-50.

10. **Tanumihardjo SA, Cheng JC, Permaesih D, Muherdiyantiningsih, Rustan E, Muhilal, Karyadi D and JA Olson** Refinement of the modified-relative-

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dose-response test as a method for assessing vitamin A status in a field setting: experience with Indonesian children. *Am. J. Clin. Nutr.* 1996; **64:** 966-971.

11. **Tchum SK, Tanumihardjo SA, Newton S, de Benoist B, Owusu-Agyei S, Arthur FK and A Tetteh** Evaluation of vitamin A supplementation regimens in Ghanaian postpartum mothers with the use of the modified-relative-dose-response test. *Am. J. Clin. Nutr.* 2006: **84;** 1344-9.

12. **De Pee S and CE West** Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. *Eur. J. Clin. Nutr.* 1996; **50** Suppl 3: S38-353.

13. **Williams AW, Boileau TW and JW Erdman Jr** Factors influencing the uptake and absorption of carotenoids. *Proc. Soc. Exp. Biol. Med.*1998; **218:** 106-8.

14. **Tanumihardjo SA** Factors influencing the conversion of carotenoids to retinol: bioavailability to bioconversion to bioefficacy. *Int. J. Vitam. Nutr. Res.* 2002; **72:** 40-45.

15. **Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ and AJ Benade** Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *Am. J. Clin. Nutr.* 2005; **81:** 1080-1087.

16. **Smith FI and P Eyzaguirre** African leafy vegetables: their role in the world health organization's global fruit and vegetables initiative. *African J. of Food Agriculture Nutrition and Development Online* 2007: 7(3). Available from www.ajfand.net Accessed 15 October 2007.

17. **Imbamba SK** Leaf protein content of some Kenya vegetables. *East African Agriculture and Forestry Journal* 1973; **38:** 246-251.

18. **Adams CA and M Richardson** Nutritive value of foods. USDA Home and Garden Bulletin 72, Washington DC, 1977.

19. **Gomez MI** Carotene content of some green leaf vegetables of Kenya and effects of dehydration and storage on carotene retention. *J. Plant Food* 1981; **3:** 231-244.

20. **Gomez MI** The evaluation of fruit and vegetable resources in the Machakos District in relation to seasonal deficient and micronutrient deficiencies.

Technical Report IDRC 39, Ottawa, Canada, 1982.

21. **Schippers RR** African indigenous vegetables: an overview of the cultivated species. Natural Resources Institute / ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK, 2000.

22. **International Vitamin A Consultative Group, IVACG** Maternal night blindness: extent and associated risk factors. IVACG Statement. Washington, DC, 1997

23. **Khatry SK, Christian P, West KP and J Katz** Effect of maternal vitamin A or beta-carotene supplementation on incidence of birth defects among Nepalese infants. **In:** Report of the XVIII IVACG Meeting, Cairo, 1997. Washington, DC: IVACG, 1998: 87.

24. **Semba RD, Miotti P and JD Chiphangwi** Maternal vitamin A deficiency and infant mortality in Malawi. J. Trop. Paediatr. 1998; **44:** 232-234.

25. **Wolf G** The regulation of retinoic acid formation. Nutr. Revs. 1996; **54:** 182-184.

26. **World Health Organization** Global prevalence of vitamin A deficiency. Micronutrient Deficiency Information System (MDIS) Working Paper #2, WHO/NUT/95.3. Geneva: WHO, 1995.

27. **West KP Jr, Katz J and SK Khatry**. Double blind, cluster randomized trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. BMJ 1999; **318:** 570-5

28. **Gebhardt SE and GT Robin** Nutritive Value of Foods. U.S. Department of Agriculture, Agricultural Research Service, Home and Garden Bulletin. 2002; **72:** 7.

29. **Shantz EM and JH Brinkman** Biological activity of pure vitamin A_2. *J. Biol. Chem.* 1950; **183:** 467-471.

30. **Verhoef H and CE West** Validity of the relative-dose-response test and the modified-relative-dose-response test as indicators of vitamin A stores in liver. *Am. J. Clin. Nutr.* 2005; **81:** 835-839.

31. **Rice AL, Stoltzfus RJ, de Francisco A, Chakraborty J, Kjolhede CL and MA Wahed** Maternal vitamin A or beta-carotene supplementation in lactating

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Bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. *J. Nutr.* 1999; **129:** 356-365.

32. D**e Pee S, West CE, Muhilal, Karyadi D and JG Hautvast** Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. *Lancet* 1995; **346:** 75-81.

33. **National Research Council and National Academy of Sciences** Recommended dietary allowances, 10th ed. (Report of the Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences). Washington, DC: National Academy Press. 1989: 85.

34. **Tanumihardjo SA** Can lack of improvement in vitamin A status indicators be explained by little or no overall change in vitamin A status of humans? *J. Nutr.* 2001; **131:** 3316-3318.

35. **Arroyave G** Alternative strategies with emphasis on food fortification. **In:** West KP and A Sommer (Eds). Delivery of oral doses of vitamin A to prevent vitamin A deficiency and nutritional blindness . A state-of-the-art review. Geneva: UN ACC Sub-committee on Nutrition. 1987: 87-91.

36. **West KP and A Sommer** Delivery of oral doses of vitamin A to prevent vitamin A deficiency and nutritional blindness: A state-of-the-art review. Geneva: UN ACC Subcommittee on Nutrition. 1987.

37. **Underwood BA** Vitamin A prophylaxis programs in developing countries: past experiences and future prospects. *Nutr. Rev*. 1990; **48:** 265-274.

38. **De Pee S, West CE, Permaesih D, Martuti S, Muhilal and JG Hautvast** Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and beta-carotene in schoolchildren in Indonesia. *Am. J. Clin. Nutr*. 1998; **68:** 1058-1067.

39. **Haskell MJ, Jamil KM, Hassan F, Peerson JM, Hossain MI, Fuchs GJ and KH Brown** 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*. 2004; **80:** 705-714.

40. **Tanumihardjo SA** Food-based approaches for ensuring adequate vitamin A nutrition. *Comp. Rev. Food Sci. Food Safety*. 2008; **7:** 373-381.

41. **Howe JA and SA Tanumihardjo** Carotenoid-biofortified maize maintains

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adequate vitamin A status in Mongolian gerbils. *J. Nutr*. 2006; **136:** 2562-2567.

42. **Tanumihardjo SA and Z Yang** Carotenoids: epidemiology of health effects. **In:** Caballero B, Allen L and A Prentice (Eds). Encyclopedia of Human Nutrition, 2nd ed. Oxford: Elsevier, 2005: 339-345.

43. **Penniston KL and SA Tanumihardjo** The acute and chronic toxic effects of vitamin A. *Am. J. Clin. Nutr*. 2006; **83:** 191-201.