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카로텐 공급이 정상식이 여성과 채식주의 여성의 혈청 카로텐 및 레티놀 수준에 미치는 영향*

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Influence of Carotene Supplementation on Serum Carotene and Retinol Levels in Lactoovovegetarian and Nonvegetarian Women

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국 문 초 록

건강한 정상식이 여성과 낙농제품을 섭취하는 채식주의 여성을 대상으로 당근쥬스를 이용한 알파와 베타 카로텐의 공급이 혈청 알파와 베타 카로텐, 총카로테노이드 및 레티놀 수준에 미치는 영향을 연구하였다. 두 주간의 실험기간동안 3일간의 식이기록과 비타민 A 섭취에 대한 기록을 하였으며, 실험기간동안 카로텐함량이 많은 식품의 섭취를 제한하였다. 그 결과, 당근쥬스 공급은 정상식이 여성의 혈청 알파와 베타 카로텐 및 총 카로테노이드 농도를 유의성 있게 상승시켰으며, 채식주의자에게선 알파와 베타 카로텐 농도만이 증가되었다. 식이 알파와 베타 카로텐의 영향은 채식주의 여성에서보다 정상식이 여성의 혈청에 더 큰 영향을 주었다. 카로텐 섭취량을 감소시킨 결과, 상승된 혈청 카로텐 수준에 있어 정상식이 여성이나 채식주의 여성 사이에 차이가 없었다. 혈청 레티놀 농도는 카로텐 공급에 영향 받지 않았으며, 실험 기간동안 일정수준을 유지하였다. 이상의 결과로 혈청 총카로테노이드 수준은 알파와 베타 카로텐 같은 각각의 카로텐의 혈청 지표가 될 수 없으며, 알파와 베타 카로텐의 경우, 식습관에 관계없이 식이 카로텐이 혈청 카로텐 수준에 반영됨을 시사해 준다.

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INTRODUCTION

Vegetarianism has not always been considered as acceptable for optimum nutrition because of limited animal food sources and particular attention needs to pregnant and lactating women and to children. However, recent investigations showed that a diet oriented more forward plant foods could be acceptable and even beneficial for health and preventing chronic diseases[1)2)]. The consumption of large amounts of fresh fruit and fresh green vegetables is reported in association with reduced risks of death from cerebrovascular disease[3)]

Numerous epidemiological studies suggest that diets high in β-carotene demonstrated a protective effect against cancer[4)5)]. Carotenoids do inhibit carcinogenesis in specific animal model[6)7)]. It is thought that β-carotene acts as a potent free radical scavenger and quencher of singlet or excited oxygen and free radicals that might lead to oxidation thereby retarding carcinogenesis [8)].

Human blood contains various kinds of carotenoids such as lutein, zeaxanthin, cryptoxanthin, lycopene, α-and, β-carotene[9)]. Since plasma carotenoids reflect dietary carotenoids and β-carotene uptake is nearly linear to concentration, diffusion-limited mechanism is proposed for absorption of carotenoid[10)]. Presences of antioxidants, tocopherols, ascorbic acid, protein and fat in deit were known to be factors affecting absorption and utilization of carotenoids[10)11)]. Roels [12)] reported that although diet contained adequate amounts of carotenoids, absorption was so inefficient that vitamin A dificiency occurred frequently in Central Africa. Serum carotenoid levels tend to be much higher among vegans than among other nonvegetarians although vitamin A values are similar[13)]. Therefore, it is necessary to investigate whether supplementary carotenes are converted into retinol, reflecting in blood levels or cleaved into apocarotenoids which may possess other biological functions, preventing body from various chorc diseass.

This stury was conducted to determine the effect of carotene supplementation on serum α-and β-carotene, total carotenoid and retinol levels in lactoovovegetarian and nonvegetarian women and to monitor the changes in these serum components after consumption reduced.

MATERIALS AND METHODS

Subjects

Five healthy nonvegetarian women aged 21-35 yr(mean age 26) and five lactoovovegetarian aged 23-48yr(mean age 30) living in the state of Rhode Island(U.S.A.) were assigned to the Protocol for the study. After registration all participants were screened for determination of eligibility.

Indeviduals were considered eligible if they had no illness or pregnancy and had no history of hepatic, renal, hematological or intestinal diseases. Individuals with body weights exceeding $\pm 20\%$ of desirable body weight were excluded. The participants entered the study after signing an appropriate informed consent approved by the University Committee on Research Involving Human Subjects at the University of Rhode Island.

Diet considerations

During study period, the subjects consumed diets for their own choice and were asked to complete three day dietary records. To minimize

variations in carotene intake from large differences in consuption of food sources containing β-carotene, a diet book was provided with a list of foods exceptionally high in carotenoid that were to be avoided. Food frequency questionnaire was also used to determine the average daily intake of carotene and preformed vitamin A during 2 week study period. The questionnaire consisted of a list of 50 carotenoid - or vitamin A-containing fruits, vegetables, their juices, dairy products and animal meats. Participants were asked to indicate the average frequency and the amont they consumed of each food. Frequency questionnaire consisted of five categories depending on the consumption of each food ; two to three times per day, once a day, two to three times per week, once a week and never taken. Dietary analysis was made with IBM / PC nutritionist (N-squared computing analytic software, Silverton, OR) based on Home and Garden Bulletin No. 72[14].

Carotene supply

Carrot juice was used as a carotene supplement for several reasons. Carrot juice is a natural source of carotene with a fine texture and the absorption of carotene is promoted if food is of fine texture[15]. Carrot contains a relatively high amounts of α-carotene, both of which are known to have vitamin A activity. Frozen carrot juice(Escondido Juice Co.Ca)was consumed after thawing and storing under refrigeration. They did not contain any preservatives or added water. The α-and β-carotene content of carrot juice were analyzed by HPLC before the study began and contents of α-and β-carotene were 42.6μg / ml and 72.9μg / ml, respectively small amounts of lycopene and unknown carotene were also detected[16]

Carotene supplementation schedules and blood collection

All participants consumed two containers of carrot juice daily for one week and then took one container every other day for another one week. One bottle of carrot juice(240ml) contains approximately 11mg of α-carotene and 17.5mg of β-carotene, respectively. Before the study and after 1 week and 2 week during the study, heparinized blood samples were collected, immediately protected from light, placed on ice, centrifuged to separate serum and stored at -20°C.

Analytical method for determination of carotene and retinol

Crystalline α-, β-carotene and retinol were purchased from Sigma Chemical Co.(St. Louis, MO). α-and β-carotene were purified by column chromatography[17] and recrystallized using a modification of the procedure of Britton and Goodwin[18]. All reagents were HPLC grade. All aqueous and organic solvents were filtered through a 0.45μm membrane filter and degassed before use. Acetone and petroleum ether were freshly distilled before use.

A Waters(Milford, MA) 6000A solvent delivery system, Perkin-Elmer LC085 detector(-Norwalk, CT) and LC autocontrol connected with a Perkin-Elmer LC 1100 Laboratory computing integrator were used. The column was a Merck RP-C 18, 4.6 by 25cm(E. Merck, Darmstadt, F.R. Germany) with 10μ particle size.

Total carotenoids were measured spectrophotometrically and α-and β-carotene were determined by HPLC. Serum(0.7ml) was placed in lml methanolic KOH(lmole / L solution of

Table 1. HPLC chromatographic condition

Condition	Carotenoid	Retinol
Column	Merck RP-C 18, 10 μm (25 × 4.5 cm, i.d.)	Merck RP-C 18, 10μm (25 × 4.6 cm, i.d.)
Mobile phase	acetonitrile/methanol/ acetone (40 : 40 : 20)	acetonitrile/methanol (50 : 50)
Flow rate	2 ml/min	1 ml/min
Pressure	1500 psi	700 psi
Detector wavelength	450 nm	325 nm
Detection sensitivity	0.01 A.U.F.S.	0.02 A.U.F.S.
Temperature	ambient	ambient

KOH in absolute methanol) and shaked in water bath(60°C) for 30min. After cooling, mixture was extracted with 2ml of petroleum ether(PE) and centrifuged at 1000xg, 10 min. Extraction procedrue was repeated and 100μl of combined PE extract was filtered through 0.2μm membrane filter and used for analysis. For retinol level, 0.7ml of serum was placed in 0.7ml of methanol and extracted with 1.4ml of methylene chloride, vortexing and centrifuged at 1000xg for 10min. The bottom layer was collected, evaporated under nitrogen and dissolved in chromatographic solvent system[19]. The chromatographic conditions are shown in Table 1. Retinyl palmitate was only used for the detection and not for the detection and not for quantitation. Quantitation was performed with standard curves generated by comparing quantities of standards vs. integrated peak areas.

Statistical analysis

Data are presented as mean and standard deviation(S.D.) of the levels at baseline, week 1 and week 2 of blood specimens. Student's t-test was used to compared the changes in blood levels among the subjects between the groups.

RESULTS AND DISCUSSION

Daily intakes of nutrients and vitamin A during study period

As shown in Table 2, there were no significant differences between nonvegetarian and lactoovovegetarian in daily intakes of nutrients during 2 week study period. Even energy intakes of participant did not reach to U.S. RDA level[20], values Were similar to those reported by Kelsay et al.[21] They showed that energy intakes of American female vegetarian and nonvegetarians were 1742Kcal and 1656Kcal. In fourteen Korean college women(mean age 19 yr), energy intake was 1542Kcal which was also lower than Korean RDA[22]. Our result and other studies demonstrate that female persons who are Koreans or Americans seem to take lower energy than recommended dietary allowances. However, it should be cnsidered that the subjects used in the study represent relatively small and restricted populations. In this study, lactoovovegetarian had slightly higher percent energy from carbohydrate. Dietary composition of carbohydrate, protein and fat by

Table 2. Mean daily intakes of selected nutrients and vitamin A by lactoovovegetarians and nonvegetarians.

	Nonvegetarian	Lactoovovegetarian
From 3-d diet records;		
Energy (Kcal)	1785±527	1694 ±258
Carbohydrate (g)	215.0±34.1	223.4±29.3
(% Kcal)	49.2±1.5	53.8±1.5
Protein (g)	66.9±54.2	60.8±35.0
(% Kcal)	15.0±0.6	12.6±0.5
Fat (g)	72.9±40.0	62.0±12.1
% Kcal)	36.8±1.3	33.6±12.1
Thiamin (mg)	1.3±0.4	1.2±0.5
Riboflavin (mg)	1.5±0.9	1.3±0.3
Niacin (mg)	21.4±7.0	20.4±8.5
Ascorbic acid (mg)	90.0±57.0	127 ±96.1
Calcium (mg)	835 ±658	505 ±391
Iron (mg)	15.2±7.4	14.0±5.0
From Food frequency questionnaire;		
Carotenoids (RE)	139± 76	179±150
Preformed vit.A (RE)	427±222	275± 95
Total vit.A (RE)	566±285	454±231

Vales represent mean ±SD. Energy and selected nutrient intakes are calculated from 3-day diet records and vitamin A intakes are analyzed from food frequency questionnaire for 2 week study period.

percentiles for energy in both groups was 48-54%, 13-15%, 34-37%. Ascorbic acid and niacin intakes were higher in both groups and Ca intake was lower than RDA in lactoovovegetarians. However, intakes of other nutrients except iron, were adequate as compared with RDA for female with similar age. Iron intakes in both group were lower than RDA[20]. For vitamin A intakes, carotenoid intakes tended to be higher in lactoovovegetarians and preformed vitamin A intakes from milk and dairy products were higher in nonvegetarian women. Total vitamin A intakes of nonvegetarians and lactoovovegetarians during the study were 566RE and 454RE, respectively which were lower than RDA of 800RE[20]. Possible reason comes from the protocol in which all participants were asked to avoid foods exceptionally high in carotenoids. Bauernfeind [23] reported that the average U.S. diet contains 1550RE of vitamin·A per day : about 350RE derived from vegetables and fruits, 600RE from fats, oils and dairy products and 600RE from meat, fish and eggs. Others[24] reported that in the U.S., the average daily intake of vitamin

Table 3. Serum levels of α-, β-carotene, total carotenoid and retinol at the beginning, week 1 and week 2 of the study.

	Nonvegetarian			Lactoovovegetarian		
	Week 0	Week 1	Week 2	Week 0	Week 1	Week 2
α-Carotene	8.6± 5.2^a	33.6± 9.4^b	41.8± 5.6^b	32.8±15.1^b	69.2±10.9^c	58.4±11.0^c
β-Carotene	22.8± 8.0^a	55.3±21.2^b	81.7±26.1^b	74.3±21.0^b	127.5±36.0^c	126.0±24.0^c
Total Carotenoid	191.9±36.2^a	285.6±48.2^b	286.2±81.2^b	340.0±55.2^b	367.7±58.0^b	354.2±55.5^b
Retinol	60.5± 8.7	61.7± 8.6	60.9± 7.9	65.5± 7.9	66.1± 7.9	66.7± 6.9

Values represent mean ±SD. Serm conponents are expressed as μg / dl, Values with different superscripts are significantly different at $p<0.05$.

A is 1260RE.

Serum levels of α-,β-carotene, total carotenoid and retinol in nonvegetarians and lactoovovegetarians.

Willet et al.[25] studied 59 generally well-nourished adults for carotenoids, vitamin E and preformed vitamin A. Mean plasma levels of total carotenoids and retinol were 179μg / dl and 55.9μg / dl. Normal plasma carotenoids value is considered as 70-300μg / dl. 30-70μg / dl are usually considered moderately decreased levels and less than 30μg / dl means severe depletion[26].

The plasma retinol concentrations of healthy normal adults are 60μg / dl for men and 50μg / dl for women[27]. In diet and cancer study[12], it is noted that in the United States, about 15% of the population has blood or liver concentrations of vitamin A below the acceptable normal limits of 20μg / dl and 40μg / dl respectively. Since nonvegetarian subjet's in this study had total carotenoid concentration of 191.9μg / dl, ranged from 120 to 245 μg / dl and retinol concentration of 60.5μg / dl, ranged from 45.4 to 80.3μg / dl, both retinol and total carotenoid concentrations of the subjects indicate well-nourished states(Table 3). Serum levels of α-, β-carotene and total carotenoid of lactoovovegetarians were 32.8μg / dl, 74.3μg / dl and 340 μg / dl, respectively. These values are 280%, 226% and 77% higher than those of nonvegetarians for α-,β-carotene and total carotenoids.

Serum retinol levels were not significantly different from levels of nonvegetarians. There are few references about the individual carotenoid concentration in human serum. Recent studies with the use of HPLC[28][29] have shown that β-carotene normally comprises 20-25% of total plasma carotenoids, with considerable variation seen from person to person. Katrangi et al.[28] reported that the ratio of α-and β-carotene to total carotenoids in the subjects varied in excess of fourfold(9.6%-43.6%). Bieri et al.[30]demonstrated that 5% of total carotenoids was α-carotene and 20.7% was β-carotene. In this study, nonvegetarian subjects had 16.4% of total carotenoids as α-carotene and β-carotene, 11.9% was β-carotene and 4.5% was α-carotene. Lactoovovegetarians had 31.4% of total carotenoid as α-carotene (9.6%) and β-carotene(21.8%). This result supports the report by Katrandgi et al.[9] who suggested that human blood contains various kinds of carotenoids such as

lutein, zeaxanthin, cryptoxanthin, lycopene, α- and β-carotene.

Effect of one week carotene supplementation on serum carotene and retinol values

After 1 week supplementation of carrot juice, serum α-and β-carotene levels increased 72% to 291% both in nonvegetarians and lactoovovetarians (Table 3). However, supplementation was more effectively reflected in nonvegetarians than in lactoovovegetarians. Serum total carotenoids levels significantly increased only in nonvegetarians and less influenced by supplements than serum α-or β-carotene. For nonvegetarians, increment in serum levels of α-and β-carotene was 57.5μg / dl after 1 week supplementation while serum total carotenoids increased 94μg / dl. This difference may come from lycopene and unknown carotene in carrot juice [16] or dietary intakes of carotenoids. Even though carotenoid consumption was restricted to all subjects in this study, we could not exclude all carotenoid supplied by foods. Human blood contains various kinds of carotenoids and α-and β-carotene comprised only 31% of total carotenoid in nonvegetarians and 53% in lactoovovegetarians even after 1 week supplementation. Lactoovovegetarians already had high levels of serum total carotenoid which was not affected by carotene supplements. Since the determination of total carotenoid concentration in clinical laboratories relied largely upon the measurement of total carotenoids via absorbance at a fixed wavelength[31][32], total carotenoid concentration by this nonspecific method could not be an indicator of specific carotene concentration in serum. It is suggested that the clinical studies in which serum α-and β-carotene levels are doubled may result in small and perhaps nonsignificant increase in total carotenoid concentration [28]. Our results support this report and in any individual the use of total carotenoid as an indicator of α-and β-carotene could be misleading.

Effect of reducing supplementation of carotene on serum carotene components in nonvegetarians and lactoovovegetarians

There were no significant differences between groups in serum levels of α-, β-carotene, total carotenoid and retinol after another one week carotene supplements with reducing amounts (240ml for every other day). However, there was a trend of serum reflection : increment in nonvegetarians and decrease in lactoovovegetarians (Table 3). For nonvegetarians, there were differences in increment in serum levels of total carotenoid and sum of α-and β-carotene. The exact reason could not be explained from this result but possibly it was contributed by differences in dietary intakes of carotenoids.

Serum retinol levels were not changed throughout the study period because serum vitamin A levels are maintained within a narrow range by liver store in adequately nourished populations and do not reflect dietary intake of vitamin A and / or possibly carotene. In a study using a rat model[33], 60-70% of the radioactively laelled β-carotene was found as retinyl ester and only about 30% as unchanged β-carotene. The retinyl ester concentration in the plasma or serum is normally very low and not quantified by HPLC unless first concentrated from very large volumes of serum, or from the sera of those supplemented with large doses of preformed- and pro-vitamin A, or individuals with liver diseases or hypervitaminosis A[34]. For further

studies, the measurement of the metabolites resulting from specific carotene, such as apo-carotenoids and retinal, retinoic acid, retinylpalmitate, retinol as well as α-retinol, by using radioactively labelling method with HPLC would be helpful to determine the metabolism of the carotene and its possible functions in the body.

SUMMARY

Normal healthy nonvegetarian and lactoovovegetarian women were studied after they consumed carrot juices as supplements. Daily supplementation resulted in significant increase in serum levels of α-, β-carotene and total carotenoid in nonvegetarians and α-and β-carotene in lactoovovegetarians. Reflection in serum α- and β-carotene was higher in nonvegetarians than lactoovovegetarians. However, serum carotene values were not significantly different between groups after continous supplementation of reducing amounts of carotenes. Serm retinol levels were not changed throughout the study period and were not significantly different between nonvegetarians and lactoovovegetarians.

The results suggest that serm total carotenoid cannot be an indicator of individual carotene such as α-or β-carotene in serum and serum carotene levels are closely related to dietary carotene intake both in nonvegetartins and lactoovovegetarians

References

1) Burr ML, Butland BK. *Heart disease in British vegetarians. Am J Clin Nutr* 48 : 830-832, 1988

2) Beilin LJ, Rouse IL, Armstrong BK, Oxon DP, Margetts BM, Vandongen RV. *Vegetarian diet and blood pressure levels : incidental or causal association. Am J Clin Nutr* 48 : 806-810, 1988

3) Acheson RM, Williams DRR. *Does consumption of fruit and vegetables protect against stroke? Lancet* 1 : 1191-1193, 1983

4) Kvale G, Bjeike E, Gart JJ. *Dietary habits and Lung cancer risk. Int J Cancer* 31 : 397-405, 1983

5) Ziegler RG, Mason TJ, Stemhagen A, Hoover R, Schoenberg JB, Gridley G, Virgo PW, Altman R, Fraumeni JF Jr. *Dietary carotene and vitamin A and risk of lung cancer among white men in New Jersey. JNCI* 73 : 1429-1435, 1984

6) Matthews-Roth MM. *Carotenoid pigment administration and delay in development of UV-B-induced tumors. Photochem Photobiol* 37 : 506-511, 1983

7) Alam BS, Alam SQ, Weir JC Jr., Gibson WA. *Chemopreventive effects of betacarotene and 13-cis-retinoic acid on salivary gland tumors. Nutr Cancer* 6 : 4-12, 1984

8) Burton GW, Ingold KU. *β-Carotene : unusual type of lipid antioxidant. Science* 224 : 569-573, 1984

9) Katrangi N, Kaplan LA, Stein EA. *Separation and quantitation of serum β-carotene and other carotenoids by HPLC. J Lipid Res* 25 : 400-406, 1984

10) Simpson RL, Chichester CO. *Metabolism and nutritional significance of carotenoids. Ann Rev Nutr* 1 : 351-374, 1984

11) Singh H, Cama HR. *Metabolism of carotenoid. J Sci Indus Res* 34 : 219-230, 1975

12) Roels OA. *Vitamin A physiology. J Am Med Assoc* 214 : 1097-1102, 1970

13) Ziegler RG, Mason TJ, Stemgahen A, Hoover R, Schoenberg JB, Gridley G, Virgo PW, Altman R, Fraumeni JF Jr. *Carotenoid intakes, vegetables, and the risk of lung cancer among white men in New Jersey. Am J Epidemiol* 123 : 1080-1093, 1986

14) Adams CF, Richardsom M. *Nutritive value of foods.* U.S. Dept. of Agr. Home & Garden Bulletin No. 72. Washington DC, 1981

15) Congden JM. *Benign carotenemia in children. Arch Dis Child* 56 : 292-294, 1981

16) Kim(Jun) H, Gerber LE. *Influence of processing on quality of carrot juice. Korean J Food Sci Technol* 20 : 683-690, 1988

17) Simpson KL, Tsou SCS, Chichester CO. *Biochemical methodology for the a ssessment of carotene. IVACG.* Nutrition Foundation Washington DC, 1982

18) Britton G, Goodwin TW. *Biosynthesis of carotenoids. Methods Enzyme* 18C : 654-706, 1971

19) Mathews-Roth MM, Stampfer MJ. *Some factors affecting determination of carotenoids in serum. Clin Chem* 30 : 459-461, 1984

20) Recommended dietary allowances. 9th ed. National Academy of Science. Washington DC, 1980

21) Kelsay JL, Frazier CW, Prather ES, Canary JJ, Clark WM, Powell AS. *Impact of variation in carbohydrate intake on mineral utilization by vegetarians. Am J Clin Nutr* 48 : 875-879, 1988

22) Yu EJ, Lim HS. *Effect of eggyolk supplementation to Korean-diet on human serum cholesterol. Korean J Nutr* 21 : 260-267, 1988

23) Bauernfeind JC. *Carotenoid, vitamin A precursors and analogs in foods and feeds. J Agr Food Chem* 20 : 456-473, 1972

24) Guthrie HA. *Introductory Nutrition. 6th ed.* Mosby College Publishing Co. St. Louis, 304-316, 1986

25) Willet WC, Stampfer MJ, Underwood BA, Taylor JO, Hennekens CH. *Validation of dietary questionnaire with plasma carotenoid and α-tocopherol levels. Am J Clin Nutr* 38 : 631-639, 1983

26) Ravel R. *Clinical Laboratory Medicine. 2nd ed.* Yearbook Medical Publishers, Inc. Chicago. 298, 1976

27) Olson JA. *Serum levels of vitamin A and carotenoids as reflectors of nutritional status. JNCI* 73 : 1439-1444, 1984

28) Katrangi N, Kaplan LA, Stein EA. *Separation and quantitation of serum β-carotene and other carotenoids by HPLC.* J Lipd Res 25 : 400-406, 1984

29) Driskell WJ, Bashor MM, Neese JW. *Beta-carotene determined in serum by liquid chromatography with internal standard. Clin Chem* 29 : 1042-1044, 1983

30) Bieri JG, Brown ED, Smith JC Jr. *Determination of individual carotenoids in human plasma by HPLC. J Liquid Chr* 8 : 473-483, 1985

31) Roels OA, Trout M. *A method for the determination of carotene and vitamin A in human blood serum, Am J Clin Nutr* 7 : 197-202, 1959

32) Targan SR, Merrill S, Schwabe AD. *Fractionation and quantitation of β-carotene and vitamin A derivatives in human serum.* Clin Chem 15 : 479-486, 1969

33) Goodman DS, Blornstrand R, Werner B, Huang HS, Shiratori T. *The intestinal Absorption and metabolism of vitamin A and β-carotene in men. J Clin Invest* 45 : 1615-1623, 1966

34) Arroyave G, Chichester CO, Flres HF, Glover J, Mejia LA, Olson JA, Simpson KL, Underwood BA. *Biochemical methodology for the assessment of vitamin A status. IVACG.* Nutrition Foundation. Washington DC, 1982