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Studies on the bioconversion of β-carotene to active vitamin A in underprivileged Guatemalan children

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The mechanism by which the rate of β-carotene conversion to active vitamin A in the enterocyte is poorly understood. It is postulated that body vitamin A status plays a very important role. Until recently, it has not been possible to detect and quantify the product of β-carotene bioconversion, i.e., retinal, in the gut, and retinyl ester, in the circulation. This study aimed at confirming that a rise in plasma retinyl palmitate concentration would occur after administration of β-carotene to underprivileged Guatemalan schoolchildren. Twenty rural children, aged 7 to 11 years, received 30 mg of pure, crystalline β-carotene in oral capsules in the fasting state. Thirteen peri-urban poor children participated as controls; each received capsules of cellulose. Standard meals with low vitamin A levels were served during the observation period. Plasma levels of β-carotene, retinol, and retinyl palmitate were determined by HPLC at baseline, 2 hr, and 24 hr after ingestion of capsules. Anthropometric and biochemical characteristics were similar in both groups at baseline. An average increase of 0.15 ± 0.05 μmol/L in retinyl esters was observed in the supplemented group at 2 hr, with a return to baseline levels at 24 hr; whereas in the control group no retinyl esters were detected at all at any time. A tendency of a greater retinyl ester response with a lower baseline circulating retinol level was observed. This is the first instance in which β-carotene bioconversion to retinyl palmitate has been quantified in children. We propose that the rise in retinyl esters after oral administration of β-carotene could be used to examine the factors that influence the rate of bioconversion of β-carotene to active vitamin A. (J. Nutr. Biochem. 8:623–628, 1997) © *Elsevier Science Inc. 1997*

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Introduction

β-carotene has a dual role in human physiology. It is a provitamin A, with the potential to yield two molecules of retinal.[1,2] Also, in its intact form, β-carotene is believed to have immunoregulatory,[3] antioxidant,[4] and antitumor[5] properties.

Orally ingested carotene can either be passed intact into the stools or taken up by intestinal mucosa. Intraluminal conditions, intestinal motility, dietary constituents, and matrix effects of the foods determine what is taken up and what is left behind in the intestinal lumen.[2,6] Within the intestinal cell, the carotene can be absorbed intact, hydrolyzed to retinol,[7,8] hydrolyzed to apo-carotenoids or other retinoids,[9] or trapped in the enterocyte until it is desquamated in the course of normal mucosal turnover. The control mechanism for the four decisions is not well understood. It is postulated that the body's reserves of vitamin A, i.e., whether the host is nutritionally adequate or is deficient, is the primary determinant of the bioconversion to the active vitamin in the intestine.[10,11] We have previously reviewed the evidence for influence or regulation of bioconversion by nutritional status in the context of the endemic nutrient deficiencies of developing country populations.[11]

Recent analytical advances in high performance liquid

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chromatography (HPLC) allow for the detection and quantification of retinyl esters. In theory, any oral β-carotene converted into retinal by the gut would appear in the circulation as retinyl ester in the postprandial chylomicra fraction. Using a population of children in which we expected to encounter a prevalence of hypovitaminosis A, our present inquiry was aimed at confirming that a rise in retinyl esters attributable to bioconversion of oral β-carotene could be detected in human plasma. In addition, we sought to identify predictors of a greater or lesser post-β-carotene response.

Methods and materials

Population

Two different groups of children were studied based on the total availability of children in each of the selected communities. An experimental group (assigned to receive β-carotene) of 20 children was examined in the town of San Pedro Carchá, which is located 200 kilometers from Guatemala City, in the northern province of Alta Verapaz. Selection of this area was made on the basis of previous findings of high prevalence of low serum retinol levels in preschool children. This group of 11 boys and 9 girls were aged 7 to 11 years (mean ± SD: 8.4 ± 1.2 y; median: 7 y). The control group (assigned to receive placebo) of 13 children was taken from Guajitos, a poor peri-urban neighborhood located to the south of downtown Guatemala City. This was a convenience-sample group, available to us as we had been performing some research in this area and we had gained confidence from the people of the area. Three boys and ten girls participated in this group; their ages ranged from 6 to 10 years (mean ± SD: 8.2 ± 1.5 y; median: 8 y).

Procedures

Parents were invited to a meeting in which they were informed about vitamin A deficiency and the objectives of the study; they were asked to permit their children to participate in the study. Those who desired to participate signed an informed consent form previously approved by the Human Subjects Committee at both CeSSIAM and the University of Arizona.

On the day of the study, the children were invited to arrive at the study site in a fasting state by 7:00 a.m. Subjects were identified and a code number was assigned to each one. Then, a 5-mL blood sample was drawn by venipuncture from each child's forearm (basal sample). Immediately thereafter, two gelatin capsules, each containing 15 mg of pure β-carotene (Hoffmann-La Roche) plus 300 mg of microcrystalline cellulose, were given to each child of the experimental group, and a similar capsule containing 330 mg of microcrystalline cellulose was given to each child of the control group. The capsules were administered along with a cup of hot chocolate (Chocolate Guerrero, Guatemala) prepared with hot water (without milk), and with 5 mL of vegetable oil (Aceite Ideal, Guatemala) added. One hour after basal blood extraction, a standard breakfast was served. A second blood sample was drawn at 2 hr after the ingestion of the challenge dose. At 12:00 p.m. a standard lunch was served, after which the children were sent home with snacks, dinner and beverages provided in a lunch box, being advised to avoid eating any food or beverage other than those provided by the study team. The standard meals served are described in (*Table 1*); their purpose was to assure that no exogenous carotene ingestion would confound the findings and to provide adequate energy and macronutrients but low levels of vitamin A. Children were invited to come the next morning to the study site in a fasting state. Once they showed up, a third (24-hr) blood sample was drawn, after which a free vitamin-A rich breakfast was served.

Table 1 Composition and nutrient content of meals served to experimental and control children

Meal	Kcal	Prot (g)	Carb (g)	Fat (g)	RE*
Breakfast	470	8.6	81.9	12.0	0.3
2 slices of white bread					
1 tbsp of honey					
2 small sweet rolls					
1 cup of chocolate[¶]					
Lunch	373	19.9	51.9	9.5	15.9
1 piece of fried chicken					
1 serving of french fries					
1 glass of soft drink					
1 roll					
Snack	397	3.0	71.0	11.2	0.0
4 sandwich type chocolate or vanilla cookies					
1 1/2 glasses of "horchata"[§]					
Dinner	902	33.8	156.6	15.6	15.9
1 piece of fried chicken					
4 slices of white bread					
4 tbsp of canned black beans paste					
2 small sweet rolls					
2 1/2 glasses of "horchata"					
TOTAL:	2142	65.3	361.4	48.3	32.1
(% energy)		12.2	67.5	20.3	

RE: retinol equivalents
[¶]Guatemalan chocolate does not contain milk.
[§]"Horchata", a beverage made of milled rice, sugar, cinnamon, and water.

Blood samples were transferred to heparin-coated glass tubes immediately after being drawn, centrifuged at 600 × g for 5 min, then plasma was separated and placed in light-protected, hermetically sealed, screw-top vials. Samples were transported on dry ice from the sites of study to CeSSIAM and stored at −20°C until they were transported, again on dry-ice, to the University of Arizona where they were stored at −70°C until chemical analysis was performed.

Chemical analysis

Before assay, 250 μL of high performance liquid chromatography (HPLC) grade methanol (Burdick & Jackson) containing apo-8 carotenal as internal standard was added to 250 μL of plasma. After vortexing for 30 seconds, hexane (0.5 mL) was added and the solution mixed with vortexing and centrifuged at 16,000 × g (Beckman Model E Microfuge, Beckman Instruments, Inc., Palo Alto, CA USA) for 2 min. The hexane layer was removed and the pellet re-extracted with an additional 0.5 mL of hexane. The hexane layers were evaporated under nitrogen and the extracted carotenoids re-suspended in 75 μl HPLC running solvent (see below). Of this, 50 μL was injected onto a Waters "Resolve" reversed-phase C18 10 μ column (4.6 mm ID × 100 mm) and eluted at a flow rate of 1.5 mL/min. HPLC analysis was performed using a Waters Model 510 pump with a Milton Roy Programmable Detector (Model SM 4000). The running solvent was acetonitrile: methylene chloride (80:20 v/v) containing 0.1% cyclohexane and 0.25 g/L BHT. Quantitation was by external standardization as previously described.[12] Limits of detection were 0.02 μmol/L (1 μg/dL), 0.35 μmol/L (10 μg/dL), and 0.03 μmol/L (1.5 μg/dL) for β-carotene, retinol and retinyl palmitate, respectively. All three compounds were expressed both as μg/dL and μmol/L.

Table 2 Baseline age and nutritional characteristics of experimental and control children. (Mean ± SD)

Characteristic	Experimental (n = 20)	Control (n = 13)
Age (years)	8.4 ± 1.2	8.2 ± 1.5
Weight (kg)	21.4 ± 3.1	24.3 ± 7.22
Height (cm)	115.3 ± 8.1	120.0 ± 9.5
Weight for height adequacy (%)	106.1 ± 9.5	105.8 ± 10.8
Plasma β-carotene (μg/dL)	2.5 ± 0.9	4.3 ± 3.0
Plasma retinol (μg/dL)	28.0 ± 7.6	31.9 ± 12.8

Data handling and analysis

A database was created on a personal computer. Derived variables, such as absolute change of retinol, β-carotene, and retinyl esters over time were calculated. Analyses included descriptive statistics. Student's t-test, and linear regression.

Results

The baseline characteristics of children in both groups are shown in (*Table 2*). Retinyl esters (retinyl palmitate) were not detectable in any control children, and only in three of 20 in the experimental group with values of 0.04, 0.03, and 0.07 μmol/L (1.87, 1.66, and 3.47 μg/dL). There were no significant differences among groups.

(*Figure 1*) shows the observed mean changes in the serum levels of β-carotene, retinol, and retinyl palmitate in both groups at 2 and 24 hr relative to the baseline values. A significant increase in circulating β-carotene occurred at 24 hr after the ingestion of the pure crystalline product ($P < 0.001$). A relative slow decrease in circulating retinol was observed in both groups at 2 hr although more marked in the placebo group, the mean changes were not statistically different; and a return to baseline values was observed at 24 hr.

Detectable levels of retinyl palmitate were not observed in the placebo group at any time. In contrast, an increase in the plasma levels of this compound was observed in the experimental group at 2 hr after ingestion of 56 mmol (30 mg) of pure crystalline β-carotene; the changes ranged from 0.07 to 0.24 μmol/L (3.55 to 12.73 μg/dL), with a mean of 0.15 ± 0.05 μmol/L (7.91 ± 2.53 μg/dL) and median of 0.14 μmol/L (7.60 μg/dL). The level returned to baseline levels at 24 hr.

(*Figure 2*) shows the regression of individual changes in retinyl palmitate at 2 hr on the baseline levels of circulating retinol. Although the slope was not statistically significant ($r = -0.29$, $P > 0.10$), with the 20 subjects studied one can observe a tendency to increasing retinyl ester responses with decreasing retinol status, supporting the hypothesis for homeostatic regulation of bioconversion of β-carotene to active vitamin A, according to individual status.

Discussion

It is well known from animal studies, and from the natural history of herbivorous species, that their entire requirement for vitamin A can be supplied from the provitamin A carotenoids in foods of plant origin.[13] A convention has been established with respect to the efficiency of bioconversion of β-carotene to retinol[1,2,6] in which it is assumed that 6 μg of dietary β-carotene will yield 1 μg of retinol, or one retinol equivalent on average for a healthy population. What is not understood for the human species is the efficiency of bioconversion of populations with increased need for vitamin A. In laboratory animal studies, indications of regulation of β-carotene bioconversion by pre-existing hepatic reserves have been found.[14–19] Dioxygenase activity increases during pregnancy, a condition associated to increased needs of vitamin A,[14] and during low dietary intakes of vitamin A;[14–16] however, such enzymatic activity decreases when animals are fed vitamin A-rich diets.[16,17] Part of the motivation of the present inquiry was to address this issue in human subjects, themselves.

Moreover, environmental, dietary, or pathologic factors can negatively influence this conversion process in the intestine. Underprivileged children live in conditions that promote intestinal parasitosis, recurrent infections, unbalanced diets, and other forms of stress and ill health. To the extent that these conditions deplete vitamin A status, they might enhance bioconversion, unless they act at the intestinal (or systemic) level to damage the mechanisms that affect the regulation of bioconversion by vitamin A status.

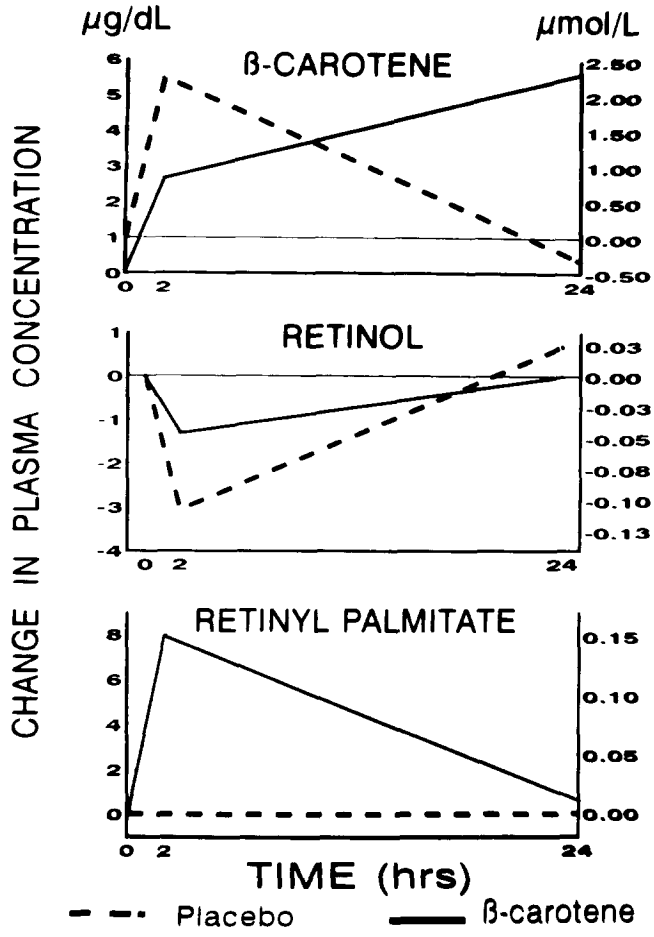

Figure 1 Observed changes in plasma β-carotene, retinol and retinyl palmitate over time in experimental and control groups.

Both the question of regulation of bioconversion of provitamin A carotenes and the question of modulation by associated factors become relevant for populations of children with a risk of vitamin A deficiency and who rely on plant sources of vitamin A and have less than ideal dietary and hygienic circumstances.

Guatemala has been known to have a prevalence of hypovitaminosis A as demonstrable by low retinol levels,[20–23] abnormal conjunctival impression cytologies,[24] and elevated relative dose responses.[25] Studies performed in the area of Alta Verapaz had shown a high prevalence of retinol levels of <0.70 μmol/L (<20 μg/dL): 50% in preschool-age children (Bulux J: unpublished findings). Although the risk of hypovitaminosis A is lessened with increasing age, the ethical and logistical considerations in a multiple blood-sampling design led us to seek the school age population from the same households. In fact, we had a range of retinol values from 0.33 to 1.41 μmol/L (9.48 to 40.30 μg/dL) in our experimental group, but only two individuals had plasma levels below the conventional cutoff of 0.70 μmol/L (20 μg/dL). Although Flores et al.[26] have argued for a critical level of 1.05 μmol/L (30 μg/dL) for preschoolers, and Carney and Russell[27] suggest the same criterion for adults, it is unclear whether school-age children should be assessed using that standard. Using the 0.70 μmol/L plasma retinol criterion, 10% of the children would fall into an abnormal classification. The schoolchildren in the control group without the oral β-carotene were taken from a peri-urban, poor neighborhood—rather than a rural area—for the sake of geographical convenience. The groups were generally comparable in measured variables including retinol status (*Table 2*).

Our experimental treatment group responded to a 56 μmol (30 mg) oral dose of β-carotene by producing a rise in retinyl esters in the range of 0.07 to 0.24 μmol/L (3.55 to 12.73 μg/dL), mean rise of 0.15 ± 0.05 μmol/L (7.91 ± 2.53 μg/dL), whereas the standard meal in the control group produced no detectable rise in retinyl esters. The only reasonable explanation, therefore, for the appearance of retinyl esters after the oral β-carotene is the bioconversion of this compound to the active vitamin. The theory behind the use of this approach has been elaborated.[11] Our empirical findings confirm in children an observation originally made in older populations.[28–30] Henderson et al.[28] administered varying doses of β-carotene to healthy volunteers under various conditions of dose, dietary fat, and timing of samples, and showed that increments of retinyl esters would appear in the peripheral circulation after the meal. Maiani et al.[29] in Italian elderly and young adults who, after receiving a dose of 28 μmol (15 mg) of β-carotene, showed increments in retinyl palmitate. Their observation can be interpreted as the actual visualization of the process of bioconversion. This approach has been carried to its most elegant status to date by van Vliet et al.[30] who dosed healthy subjects with oral 15 mg β-carotene and explored time-course of carotene and retinyl esters response in whole plasma and in the triglyceride-rich fraction. The resolution of the increment of retinyl esters was clearer if only the triglyceride-rich component is isolated for analysis, but in harmony with the aforementioned observations[28,29] and our own present observations, the response was detectable when

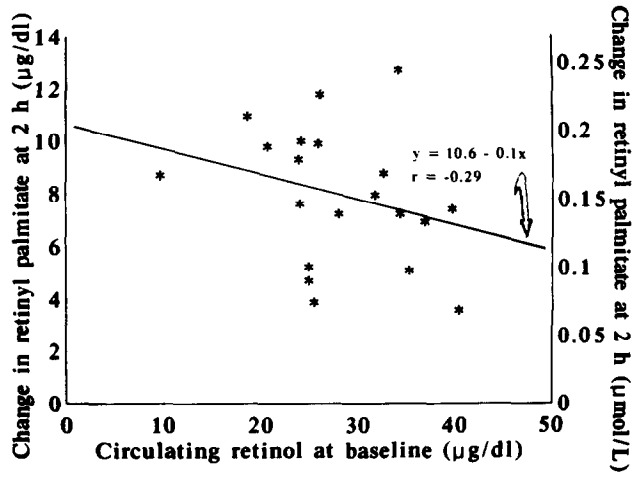

Figure 2 Linear regression of changes in plasma levels of retinyl palmitate versus baseline levels of circulating retinol in experimental children.

whole plasma alone was analyzed. Rasmussen et al.,[31] and Johnson and Russell[32] have also followed retinyl ester changes after a meal, but as it was a mixed meal containing both preformed vitamin A and carotene source, the origins of the postprandial rise in chylomicron retinoids is not clear.

A limitation in the present study, and in the generalization of the method, is the lack of a previous time-course study to determine the optimum postprandial sampling interval to maximize our detection capacity of the response of retinyl esters. This would have involved procedures with even more serial repetitions of blood extraction in young children. Thus, we chose the 2-hr interval based on empirical and intuitive grounds; it might be that some other interval provides even better resolution of the bioconversion. Parker et al.[33] using 13C-labeled β-carotene suggest a peak response of derivative 13C-retinyl esters at 4 to 5 hr. For practical purposes, however, the 2-hr interval seems to function both for detection and for the exploration of biological associations.

Among the variables measured, the best predictors of the rise in retinyl esters were the baseline retinol levels. Circulating retinol levels are recognized as an index with which to classify the population prevalence of hypovitaminosis A[34] but they have been criticized as measures of hepatic vitamin A reserves of individuals.[26,35] For the purposes of our analysis, which involved regressions, however, they had the advantage of representing a continuous variable, and the intra-individual stability is relatively high.[36] Alternative markers of vitamin A status, such as conjunctival impression cytology and relative dose response are discrete, binary variables that cannot be used easily in nonlogistic regression analyses. Despite the theoretical weakness of retinol as a marker of hepatic reserves, the most consistent interpretation of the data in (*Figure 2*), is that the degree of bioconversion of the oral β-carotene had a tendency toward a relation with retinol status as a surrogate for vitamin A status. Moreover, a series of life-event and health factors in this population of rural children not controlled for, such as frequency of recurrent gastroenteritis, intestinal parasitosis,

high fiber diet, etc. may have actually decreased the strength of inverse association that might otherwise have been expected between a marker of vitamin A status and a marker of carotene bioconversion.

Thus, based on the findings of Henderson et al.[28] and van Vliet et al.[30] in adults and Maiani et al.[29] in elderly, and the present confirmatory findings in children, we can claim a new clinical tool for the objective detection of the occurrence of bioconversion after an oral dose of β-carotene in terms of the appearance of postprandial retinyl esters, and its quantification in terms of the magnitude of retinyl esters rise. Accepting the caveats about the use of plasma retinol as a marker of vitamin A stores,[26,35] moreover, the trend toward an inverse relationship of retinyl esters rise and retinol status is consistent with the concept of regulation of enzymatic cleavage of β-carotene in the gut by the nutritional needs for the vitamin.[7,11] Further application of the retinyl esters rise after oral β-carotene dosing may allow us to elucidate the factors that influence the bioconversion of provitamin A, and to refine the conversion factor that relates ingestion of carotenoids to an equivalency in retinol equivalents of preformed vitamin A.

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References

1 Olson, J.A. (1988). Vitamin A, retinoids, and carotenoids. In: *Modern Nutrition in Health and Disease* (M.E. Shils and V.R. Young, eds.) 7th ed., pp. 292–312, Lea & Febiger, Philadelphia, PA USA

2 Underwood, B.A. (1984). Vitamin A in animal and human nutrition. In: *The Retinoids*, Vol 1. (M.B. Sporn, A.B. Roberts, and D.S. Goodman, eds.) pp. 281–392, Academic Press, New York, NY USA

3 Bendich, A. (1991). Beta-carotene and the immune response. *Proc. Nutr. Soc.* **50,** 263–274

4 Palozza, P. and Krinsky, N.I. (1992). β-carotene and α-tocopherol are synergistic antioxidants. *Arch. Biochem. Biophys.* **297,** 184–187

5 Byers, T. and Perry, G. (1992). Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. *Annu. Rev. Nutr.* **12,** 139–159

6 DePee, S., Nyugen, C.K., and West, C.E. (1996). Dietary carotenoids to combat vitamin A deficiency? Analysis of available evidence and further research needs. Lausanne, 1995 Nestle Foundation Annual Report, pp. 65–93

7 Erdman, J. Jr. (1988). The physiologic chemistry of carotenes in man. *Clin. Nutr.* **7,** 101–106

8 Tee, E.S. (1992). Carotenoids and retinoids in human nutrition. *Crit. Rev. Food Sci. Nutr.* **31,** 103–163

9 Krinsky, N.I., Wang, X.-D., Tang, G., and Russell, R.M. (1993). Mechanism of carotenoid cleavage to retinoids. *Ann. NY Acad. Sci.* **691,** 167–176

10 Erdman, J.W., Jr., Bierer, T., and Gugger, E.T. (1993). Absorption and transport of carotenoids. *Ann. NY Acad. Sci.* **691,** 76–85

11 Solomons, N.W. and Bulux, J. (1993). Effects of nutritional status on carotene uptake and bioconversion. *Ann. NY Acad. Sci.* **691,** 96–109

12 Giuliano, A.R., Matzner, M.B., and Canfield, L.M. (1992). Assessing variability in the quantitation of carotenoids in human plasma using a variance component model. *Methods Enzymol* **213,** 391–399

13 van Vliet, T. Absorption of β-carotene and other carotenoids in humans and animal models. *Eur. J. Clin. Nutr.* **50 (Suppl 3),** S32–S37

14 Villard, V., and Bates, C.J. (1986). Carotene dioxygenase (EC1.13.11.21) activity in rat intestine: effects of vitamin A deficiency and of pregnancy. *Brit. J. Nutr.* **56,** 115–122

15 van Vliet, T., van Schaik, F., and van den Berg, H. (1992). Beta-carotene metabolism: the enzymatic cleavage to retinal. Voeding **53,** 186–190

16 van Vliet, T., van Schaik, F., van den Berg, H., and Schreurs, W.H.P. (1993). Effects of vitamin A status and β-carotene intake on dioxygenase activity in rat intestins. *Ann. NY Acad. Sci.* **691,** 220–222

17 Biesalski, H.K., and Weiser, H. (1994). β-carotene supplements cannot meet all vitamin A requirements of vitamin A-deficient rats. *Ann. NY Acad. Sci.* **691,** 216–219

18 van, Vliet T. (1995). Intestinal absorption and cleavage of β-carotene in rat, hamster and human models. Ph.D. thesis. University of Amsterdam

19 Lederman, J.D., Overton, K.M., Hofmann, N.E., Moore, B.J., Thornton, K., and Erdman, J.W., Jr. Bioavailability of beta-carotene and vitamin A from pelleted diet or single oral dose and effects of vitamin A status on conversion of beta-carotene to vitamin A in the ferret. *J. Nutr.* (in press)

20 Arroyave, G., Aguilar, J.R., Flores, M., and Guzman, M.A. (1979). Evaluation of sugar fortification with vitamin A at the national level. Pan American Health Organization Scientific Publication No 384., PAHO Washington, D.C., USA

21 Arroyave, G., Mejia, L.A., and Aguilar J.R. (1981). The effects of vitamin A fortification of sugar on the serum vitamin A levels of preschool Guatemalan children: A longitudinal evaluation. *Am. J. Clin. Nutr.* **34,** 41–49

22 Solomons, N.W., Bulux, J., Guerrero A.M., de Portocarrero, L., Quan de Serrano, J., Quiñonez, J., Rosas, A.M., Vasquez, A., Zepeda, E., Brown, K.H., Forman, M., Gadomski, A., Kjolhede, C., Morrow, F., and Russell, R.M. (1989). Vitamin A en áreas urbanas marginales de la Capital de Guatemala. [Vitamin A status in urban marginal areas in Guatemala City] *Rev. Chil. Nutr.* **17(Suppl),** 41–45

23 Solomons, N.W., Haskell, M., Bulux, J., and Molina S. (1990). Nutritional and health conditions of the peri-urban pre-school and child populations of Guatemala City. In: *Urban Nutrition: Pre-School Children* (W.I.J. Aalbersberg, ed.). The Foundation for the Advancement of the Knowledge of the Nutrition of Mother and Child in Developing Countries, The Netherlands

24 Kjolhede, C.L., Gadomski, A.M., Wittpenn, J., Bulux, J., Rosas, A.R., Solomons, N.W., Brown, K.H., and Forman, M.R. (1989). Conjunctival impression cytology: feasibility of a field trial to detect subclinical vitamin A deficiency. *Am. J. Clin. Nutr.* **49,** 490–494

25 Gadomski, A.M., Kjolhede, C.L., Wittpenn, J., Bulux, J., Rosas, A.R., and Forman, M.R. (1989). Conjunctival impression cytology (CIC) to detect subclinical vitamin A deficiency: Comparison of CIC with biochemical assessments. Am J Clin Nutr **49,** 495–500

26 Flores, H., Campos, F., Araujo, C.R.C., Underwood, B.A. (1984). Assessment of marginal vitamin A deficiency in Brazilian children using the relative dose response procedure. *Am. J. Clin. Nutr.* **40,** 1281–1289

27 Carney, E.A., and Russell, R.M. (1980). Correlation of dark adaptation test results with serum vitamin A levels in diseased adults. *J. Nutr.* **110,** 552–557

28 Henderson, C.T., Mobarhan, S., Bowen, P., Stacewicz-Saputzankis, M., Langenberg, P., Kiani, R., Lucchesi, D., and Sugerman, S. (1989). Normal serum response to oral β-carotene in humans. *J. Am. Coll. Nutr.* **8,** 625–635

29 Maiani, G., Mobarhan, S., Ceccanti, M., Ranaldi, L., Gettner, S., Bowen, P., Friedman, H., DeLorenzo, A., and Ferro-Luzzi, A. (1989). β-carotene serum response in young and elderly females. *Eur. J. Clin. Nutr.* **43,** 749–761

30 van Vliet, T., Schreurs, W.H.P., and van den Berg, H. (1995). Intestinal β-carotene absorption and cleavage in men: response of β-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single dose of β-carotene. *Am. J. Clin. Nutr.* **62,** 110–116

31 Rasmussen, H.M., Dallal, G.E., Phelan, E., and Russell, R.M. (1991). Serum concentrations of retinol and retinyl esters in adults in

response to mixed vitamin A and carotenoids containing meals. *J. Am. Coll. Nutr.* **10,** 460–465

32 Johnson, E.J., and Russell, R.M. (1992). Distribution of orally administered β-carotene among lipoproteins in healthy men. *Am. J. Clin. Nutr.* **56,** 128–135

33 Parker, R.S., Swanson, J.E., Marmor, B., Goodman, K.J., Spielman, A.B., Brenna, J.T., Vierec, S.M., and Canfield, W.K. (1994). Study of metabolism in humans using 13C-β-carotene and high precision isotope ratio mass spectrometry. *Ann. NY Acad. Sci.* **691,** 86–95

34 Arroyave, G., Chichester, C.O., Flores, H., Glover, J., Mejía, L.A., Olson, J.A., Simpson, K.L., and Underwood, B.A. (1982). Biochemical methodology for the assessment of vitamin A status. *International Vitamin A Consultative Group,* The Nutrition Foundation, Washington, D.C. USA

35 Olson, J.A. (1984). Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *JNCI* **73,** 1439–1444

36 Tangney, C.C., Shekelle, R.B., Raynor, W., Gale, M., and Betz, E.P. (1987). Intra- and interindividual variation in measurements of β-carotene, retinol and tocopherols in diet and plasma. *Am. J. Clin. Nutr.* **45,** 764–769