Original Article

Carotenoid status among preschool children with vitamin A deficiency in the Republic of the Marshall Islands

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Although carotenoids are known to be important dietary sources of vitamin A, there have been few epidemiological studies that have characterized the serum concentrations of major dietary carotenoids among preschool children with vitamin A deficiency. We conducted a population-based, cross-sectional study of serum provitamin A carotenoids (α-carotene, β-carotene, β-cryptoxanthin), non-provitamin A carotenoids (lutein/zeaxanthin, and lycopene), and retinol among 278 children, aged 1-5 y, in the Republic of the Marshall Islands. Vitamin A deficiency was defined as serum retinol <0.70 μmol/L. Geometric mean serum concentrations of carotenoids among children with and without vitamin A deficiency were 0.003 vs 0.006 μmol/L for α-carotene (*P* = 0.0017), 0.011 vs 0.023 μmol/L for β-carotene (*P* <0.0001), 0.023 vs 0.034 μmol/L for β-cryptoxanthin (*P* = 0.0075), 0.007 vs 0.012 μmol/L for lycopene (*P* = 0.037), 0.044 vs 0.052 μmol/L for lutein/zeaxanthin (*P* = 0.2), and 0.045 vs 0.074 μmol/L for total provitamin A carotenoids (*P* <0.0001) respectively. In a multivariate analysis adjusting for sex, age (Odds Ratio [O.R.] 1.44, 95% confidence interval [C.I.] 1.16-1.78), and serum provitamin A carotenoids (O.R. 0.49, 95% C.I. 0.34-0.71) were associated with vitamin A deficiency, but serum non-provitamin A carotenoids were not associated with vitamin A deficiency (O.R. 0.93, 95% C.I. 0.67-1.28). Preschool children with vitamin A deficiency in the Republic of the Marshall Islands have extremely low serum concentrations of provitamin A carotenoids and interventions are needed to improve the dietary intake of provitamin A carotenoids among Marshallese children.

Key words: α-carotene, β-carotene, β-cryptoxanthin, retinol, children, vitamin A deficiency, Republic of Marshall Islands

Introduction

Vitamin A deficiency is a leading cause of growth failure, morbidity, mortality, and blindness among preschool children in developing countries.[1] Epidemiological surveys have shown that some islands in the South and Western Pacific regions have the highest prevalence rates of clinical vitamin A deficiency that have been described in the last twelve years.[2] The rates of clinical vitamin A deficiency, i.e. nightblindness and Bitot spots, exceeded 15% in some islands.[2] Factors that have been implicated in the recent emergence of vitamin A deficiency in the Pacific region include rapid demographic change, poverty, lack of homestead food production, and replacement of traditional foods such as breadfruit, banana, taro, yam, sweet potato, pandanus, coconut, and fish with rice and sweet refined foods of low nutritional quality.[2,3] Some traditional cultivars used in the region such as yellow banana, giant swamp taro, and pandanus are carotenoid-rich.[4] The Republic of the Marshall Islands is among the nations in the Pacific region that have an extremely high rate of vitamin A deficiency.[5]

Although foods rich in carotenoids are the dominant source of vitamin A for children in developing countries, there are little epidemiological data on the relationship between serum retinol concentrations and the major serum carotenoids, α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene among children with vitamin A deficiency. In developing countries, children derive an estimated 70-90% of their total vitamin A intake from provitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin) from fruits and vegetables.[6] Serum carotenoid concentrations reflect the consumption of fruits and vegetables and are widely considered to be the best biological markers for dietary carotenoid intake.[7] In this

Table 1. Serum carotenoids and retinol among children, by age

Analyte[1]	1 to < 2 years (N = 43)	2 to < 3 years (N = 50)	3 to <4 years (N = 73)	4 to < 5 years (N = 54)	5 to < 6 years (N = 57)	P
α-carotene	0.004 (0.002, 0.007)	0.004 (0.002, 0.007)	0.004 (0.002, 0.006)	0.004 (0.002, 0.005)	0.004 (0.003, 0.006)	0.94
β-carotene	0.015 (0.010, 0.022)	0.015 (0.011, 0.019)	0.015 (0.011, 0.019)	0.013 (0.008, 0.018)	0.015 (0.011, 0.019)	0.92
β-cryptoxanthin	0.014 (0.010, 0.026)	0.027 (0.019, 0.036)	0.032 (0.024, 0.041)	0.034 (0.027, 0.043)	0.027 (0.022, 0.033)	0.0002
lycopene	0.004 (0.002, 0.007)	0.007 (0.005, 0.012)	0.010 (0.008, 0.013)	0.009 (0.005, 0.014)	0.017 (0.012, 0.024)	0.0003
lutein/zeaxanthin	0.035 (0.024, 0.052)	0.037 (0.029, 0.048)	0.052 (0.041, 0.066)	0.053 (0.038, 0.071)	0.052 (0.042, 0.065)	0.11
provitamin A carotenoids[2]	0.041 (0.030, 0.055)	0.052 (0.040, 0.069)	0.059 (0.047, 0.073)	0.058 (0.046, 0.073)	0.052 (0.043, 0.061)	0.26
non-provitamin A carotenoids[3]	0.045 (0.031, 0.066)	0.053 (0.042, 0.066)	0.069 (0.056, 0.086)	0.070 (0.052, 0.094)	0.079 (0.066, 0.097)	0.02
retinol	0.683 (0.604, 0.777)	0.590 (0.516, 0.675)	0.560 (0.511, 0.615)	0.547 (0.498, 0.601)	0.504 (0.464, 0.549)	0.0036

[1] Geometric mean (lower and upper 95% confidence limits; [2] Provitamin A carotenoids = α-carotene + β-carotene + β-cryptoxanthin;
[3] Non provitamin A carotenoids = lycopene + lutein/zeaxanthin

study, we describe the distribution of serum carotenoid concentrations and their relationships to vitamin A deficiency among children in the Republic of the Marshall Islands.

Methods

A community-based survey, the Republic of the Marshall Islands Vitamin A Deficiency Study, was conducted between November 1994 and March 1995. The total survey included 919 Marshallese children, ages 1-5, from ten atolls, who represented approximately 20% of the entire population of 1-5 year old children living the Republic of the Marshall Islands.[5] The sampling strategy for the study was based on the 1988 census of the Republic of the Marshall Islands. This census provided data on the average number of children of the target age group within each household, determined by dividing the number of children in a locality by the number of households in the same location. This number was then divided into the number of children to be sampled to obtain the number of households to be visited. Households to be visited were chosen at random. When available, the date of birth of children was ascertained from the childrens' health cards. The survey team consisted of at least one Marshallese-speaking health care worker, a phlebotomist, and a medical doctor. Oral consent was obtained from a parent or guardian prior to participation in the survey, and subject anonimity was preserved by removing personal identifiers from the data set. The Ministry of Health and Envionment of the Republic of the Marshall Islands supported the project and assisted with the planning and development of this evaluation.

Blood samples were obtained by venipuncture. Venous blood samples were immediately wrapped in aluminum foil and stored at 4°C until centrifugation (200 x g, 10 minutes, room temperature) in a local laboratory. Aliquots of serum were made in cryovials, and samples were placed immediately in liquid nitrogen. Serum samples were kept in liquid nitrogen or at -70°C until the time of laboratory analysis, which occurred within two years of the survey. Serum retinol and carotenoids are stable in samples that have been stored at -70°C for more than fifteen years.[8] Serum retinol and carotenoids were measured using reversed-phase high performance liquid chromatography.[5,9] Quality control reference standards (Standard Reference Material 968C, National Institute of Standards and Technology, Gaithersburg, MD) were evaluated to ensure the quality of our measurements. Pooled human standards were used to measure intra- and inter-assay coefficients of variation in laboratory ana-lyses. For serum retinol, the within-assay and between-assay coefficients of variation were 3% and 8%, respectively. For α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene, the within-assay coefficients of variation were 7.9% and 6.3%, and 5.9%, 8.8% and 5.9%, respectively. The between assay coefficients of variation were 11.3% for β-carotene and 6.2% for total carotenoids. The study protocol was approved by the institutional review board of the Pacific Health Research Institute of Hawaii and the Ministry of Health and Environment of the Republic of the Marshall Islands. The study protocol conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Groups were compared using Student's *t*-test for continuous variables where appropriate, and categorical variables were compared using chi-square or exact tests. Vitamin A deficiency was considered consistent with serum retinol <0.70 μmol/L.[10] Non-parametric tests were used to examine trend across age categories. Provitamin A carotenoids were defined as the sum of α-carotene, β-carotene, and β-cryptoxanthin in μmol/L, and non-provitamin A carotenoids were defined as the sum of lutein/zeaxanthin and lycopene in μmol/L. Univariate and multivariate logistic regression analyses were used to estimate the relative risks of factors associated with vitamin A deficiency. Regression coefficients were converted to odds ratios (OR), and the confidence intervals (CI) for the odds ratios were derived from the standard error estimates of the regression coefficients.

Results

Out of the 919 children in the survey, 278 children had serum carotenoid concentrations measured. The mean age (± SD) of the 278 children was 3.1 ± 1.3 years, and there were 137 boys and 141 girls. The subsample of children involved in the present study was compared by age and

sex with the children who were not selected for the study. There were no significant differences by age, sex, or retinol concentrations (data not shown). The serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene among children by age in years is shown in Table 1. Serum β-cryptoxanthin and lycopene increased significantly with increasing age, whereas serum retinol concentrations decreased with increasing age. There were no significant differences in α-carotene, β-carotene, lutein/zeaxanthin, or provitamin A carotenoid concentrations with age. Serum carotenoid concentrations were compared between children with and without vitamin A deficiency (Table 2). Children with vitamin A deficiency had significantly lower geometric mean serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and provitamin A carotenoids compared to children without vitamin A deficiency. There were no significant differences in geometric mean serum concentrations of lutein/zeaxanthin or non-provitamin A carotenoids between children with and without vitamin A deficiency. Serum carotenoid concentrations were compared between boys and girls, and there were no significant differences in geometric mean serum concentrations of any of the carotenoids by sex (data not shown). Geometric mean serum retinol concentrations (SD) were 0.541 (0.507, 0.578) and 0.596 (0.557, 0.636) μmol/L among boys and girls, respectively (P = 0.04). Univariate and multivariate analyses were conducted in order to examine risk factors associated with vitamin A deficiency (Table 3). In univariate analyses, lower concentrations of serum pro-vitamin A carotenoids and increasing age were significantly associated with an increased risk of vitamin A deficiency. In multivariate analyses adjusting for sex and age, serum provitamin A

carotenoids were associated with vitamin A deficiency (P <0.0001) whereas non-provitamin A carotenoids were not associated with vitamin A deficiency (P = 0.65).

Discussion
To our knowledge, this is the first study to describe the major serum carotenoids among preschool children with and without vitamin A deficiency in a developing country. These data suggest that serum carotenoid concentrations are profoundly low among preschool children in the Republic of the Marshall Islands. There has been a paucity of studies of serum carotenoids among children in the scientific literature.[11] It is notable that the geometric mean carotenoid concentrations for α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene among Marshallese children was below the 5th percentile for all of these major blood carotenoids in a slightly older group of children in the US. Among 839 children aged 6-7 years who participated in the third National Health and Nutrition Examination Survey in the US, 1988-1994, the 5th percentiles in μmol/L were 0.012 for α-carotene, 0.124 for β-carotene, 0.083 for β-cryptoxanthin, 0.177 for lutein/zeaxanthin, and 0.167 for lycopene.[12] The geometric mean concentrations of serum carotenoids of Marshallese children were also below the 25th percentile for the respective serum carotenoids among children in Belize. Among 493 children in Belize, aged 3-9 years, the 25th percentiles in μmol/L were 0.056 for α-carotene, 0.130 for β-carotene, 0.090 for β-cryptoxanthin, 0.176 for lutein/zeaxanthin, and 0.056 for lycopene.[11] The geometric mean concentrations of serum carotenoids of Marshallese children were also below that described among children with acute, uncomplicated malaria in India[13] and Uganda.[14] The serum carotenoid

Table 2. Serum carotenoid concentrations among preschool children with and without vitamin A deficiency

Carotenoid[1] (μmol/L)	Serum retinol (μmol/L)		P
	< 0.70 (N = 189)	>0.70 (N = 89)	
α-carotene	0.003 (0.002, 0.004)	0.006 (0.005, 0.008)	0.0017
β-carotene	0.011 (0.009, 0.013)	0.023 (0.019, 0.029)	0.0001
β-cryptoxanthin	0.023 (0.021, 0.027)	0.034 (0.027, 0.043)	0.0075
lycopene	0.007 (0.006, 0.010)	0.012 (0.009, 0.016)	0.037
lutein/zeaxanthin	0.044 (0.038, 0.052)	0.052 (0.043, 0.088)	0.2
provitamin A carotenoids[2]	0.045 (0.040, 0.052)	0.074 (0.062, 0.089)	0.0001
non provitamin A carotenoids[3]	0.059 (0.052, 0.069)	0.074 (0.062, 0.089)	0.082

[1] Geometric mean (lower and upper 95% confidence limits); [2] Provitamin A carotenoids = α-carotene + β-carotene + β-cryptoxanthin; [3] Non provitamin A carotenoids = lycopene + lutein/zeaxanthin

Table 3. Univariate and multivariate models of factors associated with vitamin A deficiency (retinol <0.70 μmol/L)

Characteristic	Univariate			Multivariate		
	OR	(95% CI)	P	OR	(95% CI)	P
Age (per year)	1.37	(1.13-1.66)	0.0014	1.44	(1.16-1.78)	0.0007
Male gender	1.47	(0.88-2.45)	0.13	1.28	(0.74-2.21)	0.37
Provitamin A carotenoids[1]	0.50	(0.36-0.70)	0.0001	0.49	(0.34-0.71)	0.0001
Non provitamin A carotenoids[2]	0.78	(0.59-1.03)	0.08	0.93	(0.67-1.28)	0.65

[1] Provitamin A carotenoids = α-carotene + β-carotene + β-cryptoxanthin; per log$_e$ unit increase; [2] Non provitamin A carotenoids = lycopene + lutein/zeaxanthin; per log$_e$ unit increase.

and retinol concentrations of Marshallese children were also relatively lower than those of a similar age group of children from urban slums of northeastern Brazil (M. Gamble, unpublished data). In infants from semiurban villages in Pakistan, β-carotene concentrations were undetectable, suggesting that extremely low consumption of carotenoids occurs in other high risk populations.[15] The low serum concentrations of carotenoids in Marshallese preschool children appear to reflect the dietary and demographic changes that have occurred over the last few decades in the Republic of the Marshall Islands. Traditional foods such as breadfruit, banana, taro, yam, sweet potato, pandanus, coconut, and fish have been replaced with rice, fatty foods, and refined foods of low nutritional quality, such as canned processed meat, snack foods and chips, and ramen noodles. Similar dietary changes have taken place in the region, such as in the Federated States of Micronesia.[4] A limitation of this study is that the survey and the analyses were conducted in 1994-1995, and the vitamin A and carotenoid status may have changed since the survey. Following the initial reports from this survey, a vitamin A capsule distribution program was implemented in the Republic of the Marshall Islands, but little has been done to address the intake of traditional foods among Marshallese children. Serum retinol concentrations appeared to decrease with age whereas serum provitamin A carotenoids were similar across age categories. The decline of serum retinol with increasing age is consistent with the general observation that the risk of xerophthalmia increases with increasing age among preschool children.[16-18] The intake of provitamin A carotenoids may not be sufficient to meet the needs of growing preschool children in this population with an extremely high prevalence of vitamin A deficiency. Nevertheless, serum concentrations of provitamin A carotenoids were significantly lower among children with vitamin A deficiency compared to children without vitamin A deficiency, consistent with the notion that bioavailable carotenoids reduce the risk of vitamin A deficiency. One factor that may affect the absorption of carotenoids is the amount of dietary fat that is consumed, and it is possible that a relative lack of fat may also be affecting the absorption of carotenoids among preschool children. Serum β-cryptoxanthin and lycopene concentrations increased with increasing age, and this increase might reflect an increased consumption of citrus fruit containing β-cryptoxanthin and foods containing tomato sauce or tomatoes with increasing age. A limitation of this study is that data on infectious disease morbidity was not collected for all children in the survey. The acute phase response, which can occur during infections, may depress serum retinol concentrations.[19] Infections such as malaria are not endemic in the Republic of the Marshall Islands, but diarrheal disease is a common problem among preschool children. Although the measurement of acute phase proteins has been advocated to improve the interpretation of serum retinol concentrations, elevation of acute phase proteins is common among preschool children who have clinical vitamin A deficiency, i.e night-blindness and Bitot's spots, and elevated acute phase proteins do not provide any criteria to distinguish children with clinical vitamin A deficiency from subclinical vitamin A deficiency.[20] The dietary consumption of dark green leafy vegetables and orange fruits is often advocated to combat vitamin A deficiency in developing countries,[21,22] and many studies have focused on the dietary consumption of foods rich in β-carotene.[23,24] The use of palm fruit and its products may be another effective approach in the alleviation of vitamin A deficiency.[25] Home gardening projects in the South Pacific appear to be protective against vitamin A deficiency.[26] Local measures, such as increasing homestead food production and dietary education are needed to increase the consumption of carotenoid-rich fruits and vegetables among children in the Republic of the Marshall Islands, as the present study shows low concen-trations of dietary carotenoids among these children.

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