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Relationship of serum carotenoids and retinol with anaemia among pre-school children in the northern mountainous region of Vietnam

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Abstract

Objective: To characterize the relationship between serum carotenoids, retinol and anaemia among pre-school children.
Design: A cross-sectional study was conducted in two groups: anaemic and non-anaemic. Serum levels of retinol, α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin were measured in the study subjects.
Setting: Six rural communes of Dinh Hoa, a rural and mountainous district in Thai Nguyen Province, in the northern mountainous region of Vietnam.
Subjects: A total of 682 pre-school children, aged 12–72 months, were recruited.
Results: Geometric mean serum concentrations of carotenoids (μmol/l) were 0·056 for α-carotene, 0·161 for β-carotene, 0·145 for β-cryptoxanthin, 0·078 for lycopene, 0·388 for lutein and 0·075 for zeaxanthin. The mean levels of Hb and serum retinol were 108·8 g/l and 1·02 μmol/l, respectively. The prevalence of anaemia and vitamin A deficiency was 53·7 % and 7·8 %, respectively. After adjusting for sex and stunting, serum retinol concentrations (μmol/l; OR = 2·06, 95 % CI 1·10, 3·86, $P = 0·024$) and total provitamin A carotenoids (μmol/l; OR = 1·52, 95 % CI 1·01, 2·28, $P = 0·046$) were independently associated with anaemia, but non-provitamin A carotenoids (μmol/l; OR = 0·93, 95 % CI 0·63, 1·37, $P = 0·710$) were not associated with anaemia.
Conclusions: Among pre-school children in the northern mountainous region of Vietnam, the prevalences of vitamin A deficiency and anaemia are high, and serum retinol and provitamin A carotenoids are independently associated with anaemia. Further studies are needed to determine if increased consumption of provitamin A carotenoids will reduce anaemia among pre-school children.

Keywords
Retinol
Provitamin A carotenoid
Non-provitamin A carotenoid
Anaemia
Hb
Vietnam

Vitamin A deficiency and iron-deficiency anaemia are public health problems in developing countries and are associated with growth retardation, delayed cognitive development, depressed immune responses, higher risk of xerophthalmia and blindness, and increased morbidity and mortality[1,2]. Worldwide, vitamin A deficiency affects an estimated 140 million children under 5 years of age[3].

Vitamin A deficiency is associated with anaemia. The biological mechanisms by which vitamin A deficiency contributes to anaemia is through impairment of erythropoiesis and iron metabolism and through reduced immunity and increased anaemia of infection[1]. Although many previous studies have shown an association between vitamin A deficiency and anaemia[4,5] and between serum retinol and indicators of iron status[4,6], less has been done to characterize the relationship between serum carotenoids and anaemia. Provitamin A carotenoid status reflects the intake of provitamin A carotenoids and is hence likely to be related to vitamin A status because the human body can convert these provitamin A carotenoids into the active form of vitamin A, retinol and its derivatives[7]. The relationship between carotenoids and anaemia may be important because, in developing countries, an estimated 70–90 % of the total vitamin A intake is from provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) in fruits and vegetables[8]. Therefore, foods rich in carotenoids are the main source of vitamin A for children in developing countries.

Previous studies have shown an association of vitamin A and serum provitamin A carotenoids with anaemia among pre-school children[1,9,10]. Thus, the present study

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Table 1 Characteristics of the Vietnamese pre-school children by age category

	Total		Age category (months) 12 to <24		24 to <36		36 to <48		48 to <60		60 to <72		P*
n	682		117		150		116		147		152		0·045
% female	49·3		54·7		48·7		58·6		46·3		41·4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (months)	43·0	17·5	17·7	3·4	29·8	3·9	41·6	3·9	53·8	3·7	66·0	3·7	<0·0001
Weight (kg)	12·1	2·6	8·7	1·0	10·7	1·1	12·1	1·3	13·5	1·6	15·0	1·6	<0·0001
Height (cm)	91·9	10·9	75·9	3·5	85·3	4·8	92·2	5·1	98·1	4·6	104·4	4·7	<0·0001
WAZ	−1·94	0·77	−1·99	0·82	−1·88	0·76	−1·90	0·75	−1·98	0·81	−1·96	0·70	0·705
HAZ	−1·58	1·13	−1·69	1·19	−1·26	1·27	−1·50	1·26	−1·76	0·93	−1·71	0·96	0·001
WHZ	−1·28	0·79	−1·37	0·79	−1·26	0·81	−1·25	0·79	−1·26	0·84	−1·28	0·73	0·746

WAZ, weight-for-age *Z*-score; HAZ, height-for-age *Z*-score; WHZ, weight-for-height *Z*-score.
**P* value from ANOVA.

aimed to characterize serum retinol and carotenoids and anaemia among pre-school children in a rural region of northern Vietnam.

Subjects and methods

A cross-sectional study was conducted from October to November 2003 in six rural communes of Dinh Hoa, a rural and mountainous district in Thai Nguyen Province, in the northern mountainous region of Vietnam, situated 170 km north of Hanoi. A total of 682 pre-school children aged 12–72 months were randomly chosen by cluster from lists of subjects which were provided by local health centres. The study subjects were enrolled in the study after written informed consent was obtained from the parent or guardian. Study physicians examined all children. Only those who were free from chronic, acute illness and congenital abnormality were included.

Body weight was determined to the nearest 0·1 kg, while children were minimally clothed, with a paediatric scale for children (SECA beam balance, Hamburg, Germany). Length of children <24 months of age or height of children >24 months was measured to the nearest 0·1 cm. *Z*-scores of the indicators weight-for-age (WAZ), height/length-for-age (HAZ) and weight-for-height/length (WHZ) were calculated with EPI-INFO version 3·3·2 (Centers for Disease Control and Prevention, Atlanta, GA, USA), using the National Center for Health Statistics data as a reference[11].

Blood samples were collected in the morning (07.00–11.00 hours). Three millilitres of venous blood were drawn into sterile polypropylene tubes. The tubes were kept in the dark in a cool box (0–4°C) and transported to the district health centre within 5 h. Sera were separated from cells by centrifugation at 3000*g* for 10 min at 4°C. Aliquots of serum were stored at –70°C until analysis.

Hb was determined in whole blood using the cyanmethaemoglobin method[12]. Retinol was analysed by HPLC (HPLC model LC 10ADvp; Shimadzu, Tokyo, Japan) in a darkened room at the National Institute of Nutrition, Hanoi, Vietnam[13]. Serum carotenoids were measured using reverse-phase HPLC[14] at Johns Hopkins University School of Medicine, Baltimore, MD, USA. Pooled human sera were used to measure intra- and inter-assay CV in laboratory analyses. For α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin, the within-assay and between-assay CV were 11 % and 12 %, 8 % and 8 %, 6 % and 5 %, 12 % and 8 %, 6 % and 6 %, and 15 % and 15 %, respectively. Children with Hb concentration lower than 80 g/l were referred to the health centre of the district for treatment. The Institutional Review Board of the National Institute of Nutrition and the Ministry of Health, Hanoi, Vietnam approved the study protocol.

All data were analysed using the SPSS for Windows statistical software package version 11·0 (SPSS, Inc., Chicago, IL, USA). A one-sample Kolmogorov–Smirnov test was used to assess whether the data were normally distributed. When data were not normally distributed, statistical analysis was carried out after log transformation. For continuous response variables, results are presented as means and standard deviations or as geometric means for log-transformed data. Anaemia was defined as Hb < 110 g/l for children aged 12–60 months and as Hb < 115 g/l for children aged 60–72 months[12]. The subjects who had anaemia were classified as the anaemic group while the others were designated the non-anaemic group. Vitamin A deficiency was defined as serum retinol concentration <0·70 μmol/l[13]. 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. The independent-samples *t* test and one-way ANOVA with *post hoc* analysis (Tukey's honestly significant difference) were used to determine the differences in anthropometric, carotenoid levels and biochemical indicators between anaemic and non-anaemic group, sexes and age categories. Univariate and multivariate logistic regression analyses were used to estimate the relative risks of factors associated with anaemia. Regression coefficients were converted to odds ratios,

Table 2 Vitamin A deficiency and anaemia among pre-school children in Vietnam by age category

	Total		Age category (months): 12 to <24		24 to <36		36 to <48		48 to <60		60 to <72		
	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P**
Retinol (μmol/l), mean and SD	1·02	0·26	0·96	0·24	1·06	0·23	1·06	0·34	1·04	0·25	1·00	0·23	0·009
Retinol <0·70 μmol/l, *n* and %	53	7·8	12	10·3	11	7·4	9	7·8	10	6·8	11	7·2	0·870
Hb (g/l), mean and SD	108·8	11·6	100·6	11·1	108·0	10·1	110·2	9·8	112·0	10·9	111·9	11·1	<0·0001
Anaemia, *n* and %	366	53·7	92	78·6	74	49·7	51	44·0	62	42·2	87	57·2	<0·0001

**P* value from ANOVA.

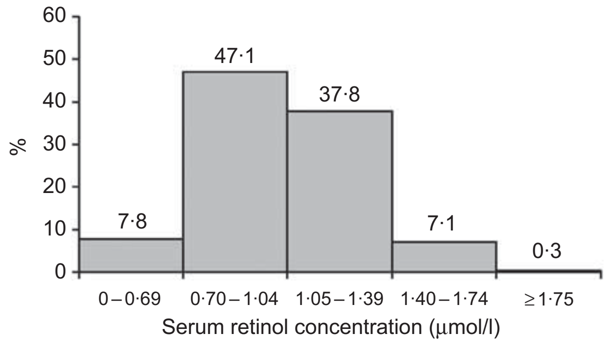

Fig. 1 Frequency distribution of serum retinol concentrations in 678 pre-school children in the northern mountainous region of Vietnam

and the confidence intervals for the odds ratios were derived from the standard error estimates of the regression coefficients. For all analyses the level of significance was $P<0·05$.

Results

A total of 682 pre-school children from the six communes were included in the study. Of these children, 676 had serum carotenoids measured, 678 had serum retinol measured and 681 children had Hb measured. The mean age of the 682 children was 43·0 (SD 17·5) months, and there were 346 boys and 336 girls. Mean WAZ was −1·94 (SD 0·77), mean HAZ was −1·58 (SD 1·13) and mean WHZ was −1·28 (SD 0·79; Table 1). The prevalence of children with WAZ, HAZ and WHZ <−2 was 46·3 % (316/682), 32·0 % (218/682) and 16·0 % (109/682), respectively. Children were divided into 12-month age categories between 12 and 72 months of age. The prevalence of underweight and wasting was not different between the five age categories ($P>0·05$). However, there were significant differences in the prevalence of stunting among the five age categories ($P=0·002$). This prevalence was highest in the age group of 48 to <60 months (38·8 %) but lowest in age group of 24 to <36 months (19·3 %).

Mean serum vitamin A of the study population was 1·02 (SD 0·26) μmol/l, and the overall prevalence of vitamin A deficiency was 7·8 % (53/678; Table 2). However, a large proportion of the children (47·1 %) had serum retinol in the range of 0·70–1·04 μmol/l, thus just above the cut-off level for vitamin A deficiency (Fig. 1). The mean Hb concentration was 108·8 (SD 11·6) g/l. Hb levels among boys (*n* 345) and girls (*n* 336) were 109·6 (SD 11·5) and 108·1 (SD 11·5) g/l, respectively, and were not statistically different ($P=0·10$). Of the total population of children, 53·7 % (366/681) were found to be anaemic (Table 2). The mean ages of children with and without anaemia were 41·0 (SD 18·7) and 45·3 (SD 15·7) months, respectively ($P=0·001$).

The serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin, provitamin A carotenoids and non-provitamin A carotenoids, among the pre-school children by age category, are shown in Table 3. Serum concentrations of α-carotene, β-carotene, lycopene and total provitamin A carotenoids were significantly higher at older age, while serum concentrations of lutein, zeaxanthin and total non-provitamin A carotenoids were significantly lower with increasing age. However, there was no trend in serum β-cryptoxanthin level by age among these subjects.

Carotenoids were classified into two main groups as provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) and non-provitamin A carotenoids (lycopene, lutein and zeaxanthin). Children with anaemia had significantly lower serum levels of retinol and geometric mean levels of α-carotene, β-carotene, β-cryptoxanthin, provitamin A carotenoids, lycopene and zeaxanthin, compared with children without anaemia. On other hand, there were no significant differences in geometric mean serum concentrations of lutein and non-provitamin A carotenoids between children with and without anaemia (Table 4).

Serum retinol and carotenoid concentrations were compared between boys and girls; there were no significant differences in mean or geometric mean serum concentrations of any of the micronutrients by gender (data not shown).

Multivariate logistic regression analysis was used to examine the relationship between retinol, provitamin A carotenoids and non-provitamin A carotenoids and anaemia (Table 5). After adjusting for gender and stunting, serum retinol concentrations (μmol/l; OR = 2·06, 95% CI 1·10, 3·86, P = 0·024) and provitamin A carotenoids (μmol/l; OR = 1·52, 95% CI 1·01, 2·28, P = 0·046) were independently associated with anaemia, but non-provitamin A carotenoids (μmol/l; OR = 0·93, 95% CI 0·63, 1·37, P = 0·710) were not associated with anaemia.

Discussion

The present study is the first to describe the major serum carotenoids among pre-school children in Vietnam, and also is one of the very few studies about carotenoid status in developing countries[15,16]. The data showed that serum carotenoid concentrations were very low among pre-school children in the north mountainous region in Vietnam. The geometric mean carotenoid concentration for α-carotene, β-carotene and β-cryptoxanthin among Vietnamese children was similar to the 25th percentiles among 839 children aged 6–7 years who participated in the third National Health and Nutrition Examination Survey in the USA between 1988 and 1994 (μmol/l: 0·030 for α-carotene, 0·206 for β-carotene and 0·131 for β-cryptoxanthin, and 5th percentile for lycopene of 0·167)[17]. The values observed among the Vietnamese children were also similar to the 25th percentile for 493 children in Belize, aged 25–115 months (μmol/l: 0·056 for

Table 3 Serum levels of carotenoids among pre-school children in Vietnam by age category

			Age category (months)										
	Total (*n* 676)		12 to <24 (*n* 116)		24 to <36 (*n* 150)		36 to <48 (*n* 113)		48 to <60 (*n* 146)		60 to <72 (*n* 151)		
Carotenoid (μmol/l)	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	*P**
α-Carotene	0·056	0·053, 0·059	0·045	0·041, 0·050	0·050	0·045, 0·055	0·061	0·054, 0·068	0·060	0·054, 0·067	0·065	0·059, 0·072	<0·0001
β-Carotene	0·161	0·153, 0·172	0·122	0·109, 0·141	0·136	0·121, 0·154	0·185	0·162, 0·211	0·182	0·158, 0·209	0·190	0·168, 0·215	<0·0001
β-Cryptoxanthin	0·145	0·134, 0·157	0·113	0·095, 0·137	0·141	0·119, 0·170	0·188	0·157, 0·225	0·144	0·119, 0·173	0·149	0·126, 0·175	0·008
Lycopene	0·078	0·073, 0·083	0·064	0·055, 0·073	0·066	0·058, 0·075	0·086	0·074, 0·101	0·083	0·072, 0·096	0·091	0·079, 0·104	<0·001
Lutein	0·388	0·370, 0·406	0·482	0·428, 0·543	0·343	0·312, 0·378	0·395	0·357, 0·437	0·379	0·339, 0·423	0·375	0·343, 0·409	<0·001
Zeaxanthin	0·075	0·071, 0·079	0·089	0·080, 0·100	0·068	0·062, 0·075	0·078	0·069, 0·088	0·073	0·066, 0·081	0·071	0·065, 0·081	<0·01
Provitamin A carotenoids	0·397	0·377, 0·423	0·308	0·274, 0·353	0·359	0·320, 0·409	0·471	0·414, 0·536	0·424	0·369, 0·487	0·441	0·392, 0·496	<0·0001
Non-provitamin A carotenoids	0·571	0·548, 0·598	0·654	0·587, 0·739	0·507	0·466, 0·555	0·591	0·534, 0·654	0·568	0·512, 0·631	0·569	0·522, 0·621	0·011

Provitamin A carotenoids = α-carotene + β-carotene + β-cryptoxanthin.
Non-provitamin A carotenoids = lycopene + lutein + zeaxanthin.
**P* value from ANOVA.

Table 4 Serum levels of micronutrients among pre-school children in Vietnam with and without anaemia

	Anaemic group		Non-anaemic group		
	n	%	*n*	%	*P**
Male gender	177	51·3	168	48·7	0·196
Female gender	189	56·3	147	43·7	0·196
	Mean	SD	Mean	SD	
Age (months)	41·0	18·7	45·3	15·7	0·001
WAZ	−2·00	0·75	−1·87	0·78	0·025
HAZ	−1·69	1·21	−1·46	1·03	0·007
WHZ	−1·29	0·80	−1·28	0·78	0·874
Hb (g/l)	100·5	8·3	118·5	6·1	<0·0001
Retinol (μmol/l)	1·00	0·24	1·05	0·28	0·017
	Geometric mean	95 % CI	Geometric mean	95 % CI	
α-Carotene (μmol/l)	0·053	0·050, 0·056	0·060	0·056, 0·064	0·012
β-Carotene (μmol/l)	0·150	0·138, 0·163	0·178	0·164, 0·193	0·006
β-Cryptoxanthin (μmol/l)	0·127	0·113, 0·142	0·169	0·152, 0·189	0·0003
Lycopene (μmol/l)	0·073	0·066, 0·080	0·084	0·076, 0·092	0·034
Lutein (μmol/l)	0·381	0·357, 0·406	0·399	0·374, 0·427	0·384
Zeaxanthin (μmol/l)	0·071	0·067, 0·075	0·080	0·075, 0·086	0·012
Provitamin A carotenoids (μmol/l)	0·364	0·336, 0·396	0·444	0·410, 0·480	0·001
Non-provitamin A carotenoids (μmol/l)	0·557	0·523, 0·592	0·595	0·559, 0·633	0·179

WAZ, weight-for-age *Z*-score; HAZ, height-for-age *Z*-score; WHZ, weight-for-height *Z*-score.
Provitamin A carotenoids = α-carotene + β-carotene + β-cryptoxanthin.
Non-provitamin A carotenoids = lycopene + lutein + zeaxanthin.
**P* value from independent-samples *t* test.

Table 5 Univariate and multivariate models of factors associated with anaemia in Vietnamese pre-school children

	Univariate model			Multivariate model		
Characteristic	OR	95 % CI	*P*	OR	95 % CI	*P*
Gender	0·82	0·61, 1·11	0·196	0·80	0·58, 1·08	0·144
Stunting	1·33	0·88, 2·02	0·179	1·20	0·78, 1·84	0·401
Serum retinol*	2·09	1·13, 3·85	0·018	2·06	1·10, 3·86	0·024
Provitamin A carotenoids†	1·53	1·07, 2·21	0·021	1·52	1·01, 2·28	0·046
Non-provitamin A carotenoids‡	1·12	0·80, 1·58	0·512	0·93	0·63, 1·37	0·710

*Serum retinol in μmol/l.
†Provitamin A carotenoids = α-carotene + β-carotene + β-cryptoxanthin (μmol/l).
‡Non-provitamin A carotenoids = lycopene + lutein + zeaxanthin (μmol/l).

α-carotene, 0·130 for β-carotene, 0·090 for β-cryptoxanthin and 0·056 for lycopene)[18]. However, the geometric mean concentrations of serum carotenoids of Vietnamese children were higher than those described among 278 children aged 1–5 years in the Marshall Islands[14]. The low serum concentrations of carotenoids in pre-school children in Vietnam may be the consequence of poor dietary intakes of lipids and carotene-rich foods[19].

The total prevalence of vitamin A deficiency was 7·8 % in the study population, which indicates that vitamin A deficiency is still a public health problem in Vietnam. Vitamin A capsule distribution programmes are the main strategy for reducing vitamin A deficiency in many developing countries[20]. Annually Vietnamese children receive high-dose vitamin A capsules through two distribution campaigns throughout the effective network of existing preventive health infrastructure at all administrative levels, with a coverage rate of 70–90 % at national level[21]. Therefore, it appears that the prevalence of vitamin A deficiency decreased from 12 % in the year 2000[22] to about 8 % in the present study, but this finding is not absolutely conclusive since the same study populations were not sampled in the two different years. The prevalence of anaemia was also extremely high in the present study, occurring in 53·7 % of the pre-school children. This prevalence of anaemia is higher than the global estimates for pre-school children (42 %) in developing countries[23]. This may be the consequence of poor iron stores of children due to too low consumption of iron-rich foods, poor iron status of the mother[19] and the high prevalence of hookworm infection in Vietnam (28·6 %)[24]. That both vitamin A deficiency and anaemia are still highly prevalent in the studied site indicates a challenge for the reduction of micronutrient deficiencies in poor areas such as mountainous regions in the country.

In agreement with results from previous studies, our study showed that serum retinol concentrations are positively correlated with Hb concentrations[1,5,10,16].

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Vitamin A deficiency impairs Hb synthesis and depresses erythropoiesis[25,26]. This was most clearly demonstrated in a clinical experiment involving anaemic adults who were depleted of vitamin A, in whom supplementation with vitamin A improved Hb levels or iron status[25]. Furthermore, several intervention studies have shown that vitamin A supplementation improves Hb response, such as a study in Malawian infants, in schoolchildren in Tanzania and in pregnant Indonesian women[27–30]. Multiple logistic regression analyses were used to determine the factors related to anaemia in the present study. Anaemic children had significantly lower levels of vitamin A, α-carotene, β-carotene, β-cryptoxanthin and provitamin A carotenoids compared with non-anaemic children, suggesting that provitamin A carotenoids play a greater role than non-provitamin A carotenoids in anaemia among these pre-school children.

In conclusion, the present study showed that the prevalence of both vitamin A deficiency and anaemia are high among pre-school children in the northern mountainous region of Vietnam. Both serum retinol and serum provitamin A carotenoids are independently associated with anaemia. Further studies are needed to determine whether increasing the intake of provitamin A carotenoids will have an impact on anaemia.

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