

Status of vitamins A and E in schoolchildren in the centre west of Tunisia: a population-based study

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Abstract

Objective: The present study was undertaken to assess the status of vitamins A and E (VA and VE, respectively) and their main determinants in Tunisian children.

Design: Cross-sectional population-based study.

Setting: Kasserine Governorate in the centre west of Tunisia.

Subjects: A total of 7407 children attending the first grade of elementary school were included. VA and VE were assessed by HPLC.

Results: The prevalence of moderate VA deficiency (VAD; $<0.70 \mu\text{mol/l}$) was 2.3% and VE deficiency (VED; $<6.97 \mu\text{mol/l}$) was 5.4%. Low status in VA ($0.70\text{--}1.05 \mu\text{mol/l}$) and VE ($6.97\text{--}11.61 \mu\text{mol/l}$) was observed in 17% and 20.2% of children, respectively. No child exhibited severe VA or VE deficiency (<0.35 and $<2.32 \mu\text{mol/l}$, respectively). The main predictors of VAD were advanced age (OR = 1.65; 95% CI 1.13, 2.41; $P = 0.05$) and sickness within the past 2 weeks (OR = 1.51; 95% CI 1.09, 2.09; $P = 0.01$). Predictors of VED were living in the peri-urban region (OR = 1.60; 95% CI 1.28, 2.01; $P < 0.001$) and sickness within the past 2 weeks (OR = 0.75; 95% CI 0.60, 0.94; $P = 0.01$).

Conclusions: Moderate VAD and VED were uncommon in Tunisian children. However, low status in VA and/or VE remains frequent. A reinforcement of the national strategies for children's nutrition and health is needed, particularly in disadvantaged regions. Supplementation of VA and VE is not necessary in Tunisia, but food fortification may be beneficial.

Keywords
Child
Malnutrition
Vitamin A deficiency
Vitamin E deficiency

Retinol and α -tocopherol, the main forms of vitamin A (VA) and vitamin E (VE) in man, are lipid-soluble compounds necessary for health. These vitamins are important for vision, growth, and immune and neurological functions^(1,2). VA deficiency (VAD) is highly prevalent and is associated with increased occurrence of infectious diseases and mortality in several developing countries, especially in sub-Saharan Africa and South-east Asia^(3–9). In addition, improving the VA status of children in these countries can reduce morbidity and mortality^(10–12). VE status has not been given the same interest as an indicator of the nutritional state in children. However, VE deficiency (VED) is thought to augment the risk for several diseases, such as CVD and cancer^(13,14).

Tunisia is a developing country located in North Africa, bordering the Mediterranean Sea. In the past 20 years, the socio-economic level, living conditions and health have improved significantly^(15,16). The under-five mortality decreased from fifty-two per 1000 live births in 1990 to

twenty-one per 1000 live births in 2007⁽¹⁷⁾. Nutrition has improved in quantity and quality. Energy supply exceeds the requirements of the population. Three food groups, cereals, oils and sweeteners, provide three-quarters of the energy availability. Consumption of meat, fish, fruit and vegetables has also increased^(18,19). Malnutrition among children has decreased significantly. Micronutrient deficiencies have decreased considerably, but anaemia due to Fe deficiency remains frequent across the country, predominantly in the west and the south⁽¹⁹⁾. VAD has been given little attention since severe deficit with clinical signs, such as night blindness and xerophthalmia, is rarely encountered. However, subclinical deficiency in VA or VE due to insufficient intake or reduced absorption could not be excluded, especially in deprived people. These deficiencies could disturb growth and immunity, and increase the risk of morbidity and mortality in children. Recently, a WHO report has estimated the prevalence of VAD at over 10% and identified VAD as a moderate public health

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problem in North Africa⁽²⁰⁾. Few studies have reported plasma VA and VE concentrations in Tunisian adults^(13,21). However, no information is available on VA and VE dietary intake or plasma concentration in Tunisian children. Therefore, it is important to assess the vitamin status of children who are at risk of deficiency before planning and carrying an intervention strategy. The present study was aimed at determining the extent of VAD and VED and their main determinants in children from a disadvantaged region of Tunisia.

Subjects and methods

Study design

A population-based study was conducted during February and March 2006, involving all the children attending the first year of elementary school (n 300) in Kasserine Governorate (around 420 000 inhabitants). Kasserine is located at the centre west of Tunisia and has the lowest socio-economic, health and environmental indicators in the country^(18,19). Children who took VA or VE supplements within the previous year were excluded. Out of 7960 children listed in the school registers, 553 were not investigated because of non-availability (n 313), stopping school (n 123) or parents' refusal (n 80). The recovery was 93.1%, which makes the sample indicative for all of the schoolchildren in the first year of elementary school in Kasserine Governorate. Among the 7407 children included, the status of VA and VE was analysed in 6677 children. Biochemical analysis was not performed in 730 children (9.8%) because of insufficient plasma quantity, severe haemolysis or poor storage. There were no significant differences according to the demographic, anthropometric and health characteristics of children who got or did not get their vitamins measured. The protocol of the survey was reviewed and approved by the Ethics Committee on Human Research of the National Institute of Nutrition (Tunis) and the Tunisian Ministry of Education. After being thoroughly informed on the purpose, requirement and procedures, all children's parents or guardians gave their free informed consent.

Study population

VA and VE measurements were performed for 6677 children, 3399 boys and 3278 girls, aged 5–7 years. According to residence area, 2811 children lived in Kasserine city (urban), 1626 children lived in settlements around the city (peri-urban) and 2240 children lived in the country (rural). All children were questioned about the occurrence of sickness (fever, cough and diarrhoea) within the past 2 weeks before the study. Anthropometric measurements were made in all children; weight and height were measured with children barefoot standing and lightly clothed. Standard normal deviates Z -score for weight-for-age (WAZ), height-for-age (HAZ) and BMI-for-age (BMIZ) were calculated based on the WHO

growth reference data for 5–19 years⁽²²⁾. WAZ was used to denote underweight as an overall indicator of malnutrition. HAZ was used as an indicator for stunting (chronic malnutrition) and BMIZ as an indicator for thinness.

Sample collection and analytical methods

Venous blood was obtained from fasting children by venepuncture and drawn into heparinized tubes. The specimens were shipped on the same day to Tunis city (300 km) and continuously kept away from light in a portable cool bag containing dry ice. Blood samples were centrifuged at 2000g for 20 min and plasma was kept frozen at -40°C until analysis (within 6 months). VA and VE were assessed by reverse phase HPLC as described by Driskell *et al.*⁽²³⁾. Briefly, plasma was deproteinized in the presence of ethanol containing butylated hydroxytoluene as antioxidant and retinol acetate as an internal standard. Vitamins were extracted with hexane and the extract was evaporated to dryness under a stream of nitrogen. The residues were dissolved in ethanol and injected into a C18 reversed-phase column (Shimpack ODS-M; Shimadzu, Kyoto, Japan). The mobile phase consisted of methanol gradient grade (Merck kGaA, Darmstadt, Germany) at a flow rate of 1.5 ml/min, and VA, retinol acetate and VE peaks were detected at 290 nm. Linearity was achieved in the range of 0.175–4.25 $\mu\text{mol/l}$ for VA and 1.16–40.6 $\mu\text{mol/l}$ for VE. The precision and the accuracy of the assay method were tested with two-level controls (Chromsystems GmbH, Munich, Germany). Long-term (n 30) imprecision (CV) was 6.1% and 5.6% at concentrations of 1.65 and 26.7 $\mu\text{mol/l}$, and 7.8% and 5.1% at concentrations of 2.60 and 32.5 $\mu\text{mol/l}$ for VA and VE, respectively. Moderate and severe deficiencies were considered for plasma VA to be <0.70 $\mu\text{mol/l}$ and <0.35 $\mu\text{mol/l}$, and for plasma VE to be <6.97 $\mu\text{mol/l}$ and <2.32 $\mu\text{mol/l}$, respectively. Low status was considered for plasma VA of 0.70–1.05 $\mu\text{mol/l}$ and plasma VE of 6.97–11.61 $\mu\text{mol/l}$ ^(24,25).

Data analysis

Statistical computations were performed using the SPSS for Windows statistical software package version 10.0 (SPSS Inc., Chicago, IL, USA). The data of each continuous variable were examined for normality using the Kolmogorov–Smirnov test. Continuous variables between groups were compared using the ANOVA test or the independent-samples t test. Differences between proportions were evaluated by Pearson's χ^2 test or Fisher's exact probability test. Mantel–Haenszel common OR were calculated as estimates of relative risk of VAD and VED for several variables. In order to identify independent predictors for VAD and VED, binary logistic regression models were applied with VAD (binary variable defined as plasma VA concentrations dichotomized at a cut-off of 7 $\mu\text{mol/l}$, with cases <7 $\mu\text{mol/l}$) and VED (binary variable defined as plasma VE concentrations dichotomized at a cut-off of 6.97 $\mu\text{mol/l}$, with cases <6.97 $\mu\text{mol/l}$) as response variables, respectively.

The independent predictors were variables that are significant at a P value <0.20 in univariate analysis, namely age group, residence area, underweight, stunting and sickness within the past 2 weeks for VAD, and residence area, stunting and sickness within the past 2 weeks for VED. Goodness-of-fit of logistic models was satisfactory. A P value <0.05 based on two-sided calculation was considered significant.

Results

The main demographic, anthropometric and nutritional characteristics of the whole population according to residence area are shown in Table 1. Compared with urban regions, HAZ and WAZ scores were significantly lower and the prevalence of underweight and stunting was higher in peri-urban and rural regions. Mean plasma

VA and VE concentrations were 1.38 (SD 0.37) $\mu\text{mol/l}$ (extreme values: 0.42 – 3.50) and 15.09 (SD 5.45) $\mu\text{mol/l}$ (extreme values: 2.55 – 75.5 ; Fig. 1). Plasma VE was significantly lower in peri-urban and rural regions than urban regions. However, plasma VA was equivalent in the three regions. The prevalence of VAD was 2.3% and VED was 5.4% . Low status was observed in 17% and 20.2% for VA and VE, respectively. No child exhibited severe VA or VE deficiency (Table 2). VAD and VED were more frequent in children living in the peri-urban regions compared with those living in urban and rural regions. VAD, but not VED, was more frequent in 7-year-old children and underweight children. A total of 2260 children (33.85%) presented a sickness within the past 2 weeks and showed higher prevalence of VAD, but lower prevalence of VED (Table 3). In multivariate analysis, VAD was significantly more frequent in 7-year-old children (multi-adjusted OR = 1.65 ; 95% CI 1.13 , 2.41 ;

Table 1 Main demographic, anthropometric and nutritional characteristics in study population according to residence area

	Residence area							
	All (n 6677)		Urban (n 2811)		Peri-urban (n 1626)		Rural (n 2240)	
Sex ratio (male/female)	50:90		49:40		51:50		52:30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	6.65	0.38	6.60	0.34	6.68	0.39	6.68	0.40
HAZ	-0.39	1.05	-0.20	1.00	-0.50	1.02**	-0.48	1.10**
WAZ	-0.65	0.87	-0.51	0.88	-0.79	0.86**	-0.74	0.85**
BMIZ	-0.67	0.99	-0.61	1.02	-0.75	1.01*	-0.68	0.93
Vitamin A ($\mu\text{mol/l}$)	1.38	0.37	1.37	0.36	1.38	0.38	1.39	0.38
Vitamin E ($\mu\text{mol/l}$)	15.09	5.13	15.93	5.53	14.56	5.67**	14.44	5.13**
	%		%		%		%	
Underweight	3.8		2.4		5.2**		4.6*	
Stunting	4.6		2.7		6.0**		5.9**	
Thinness	6.3		6.1		7.3		5.8	

HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; BMIZ, BMI-for-age Z-score.

* $P < 0.01$; ** $P < 0.001$ (comparison was made by ANOVA test for continuous variables and Pearson's χ^2 for categorical variables).

†Reference group for comparison.

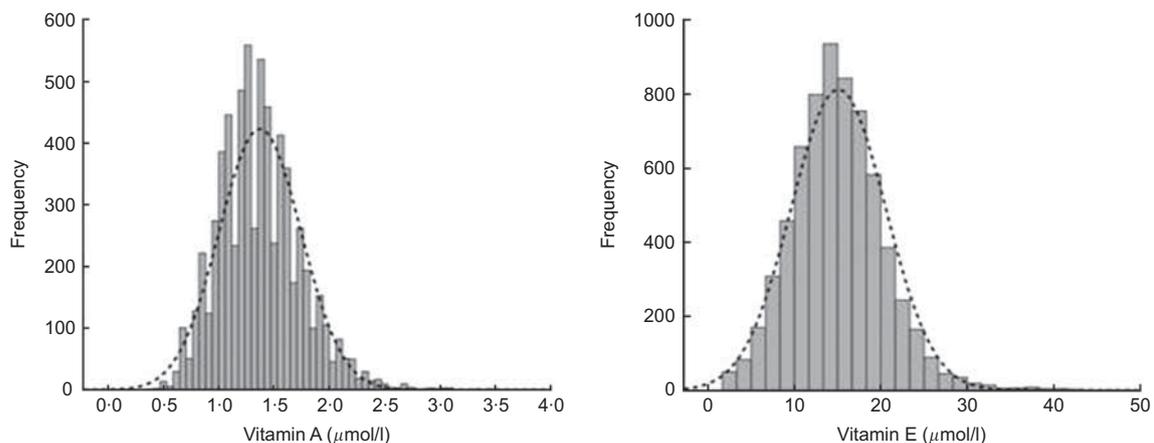


Fig. 1 Distribution of plasma vitamin A and E values in children attending the first year of elementary school in Kasserine Governorate of Tunisia (n 6677)

Table 2 Vitamin status expressed by plasma vitamin A and E concentration classes in children attending the first year of elementary school in Kasserine Governorate of Tunisia (*n* 6677)

	Plasma vitamin A		Plasma vitamin E	
	Definition	%	Definition	%
Severe deficiency ($\mu\text{mol/l}$)	<0.35	0.0	<2.32	0.0
Mild deficiency ($\mu\text{mol/l}$)	≥ 0.35 and <0.70	2.3	≥ 2.32 and <6.97	5.4
Low status ($\mu\text{mol/l}$)	≥ 0.70 and <1.05	17.0	≥ 6.97 and <11.61	20.2
Sufficient status ($\mu\text{mol/l}$)	≥ 1.05	79.7	≥ 11.61	74.4

Table 3 Prevalence and unadjusted OR with 95% CI for vitamins A and E deficiency according to demographic, health and anthropometric characteristics in children attending the first year of elementary school in Kasserine Governorate of Tunisia (*n* 6677)

Variable	<i>n</i>	Vitamin A deficiency†				Vitamin E deficiency‡			
		%	OR	95% CI	<i>P</i>	%	OR	95% CI	<i>P</i>
Gender									
Male	3399	2.1	–	–	0.40	5.3	–	–	0.61
Female	3278	2.5	1.15	0.84, 1.59		5.6	1.06	0.85, 1.64	
Age group (years)									
5	109	1.8	0.88	0.22, 3.64	0.05	7.3	1.42	0.68, 2.94	0.43
6	5482	2.1	–	–		5.6	–	–	
7	1086	3.6	1.77	1.22, 2.56		6.0	1.14	0.86, 1.50	
Residence area									
Urban	2811	2.1	–	–	0.14	4.4	–	–	<0.001
Peri-urban	1626	3.0	1.42	0.97, 2.09		7.4	1.74	1.35, 2.26	
Rural	2240	2.1	1.00	0.68, 1.47		5.3	1.21	0.93, 1.56	
Underweight									
No	6417	2.2	–	–	0.01	5.4	–	–	0.60
Yes	260	4.6	2.14	1.17, 3.91		6.2	1.15	0.68, 1.92	
Stunting									
No	6371	2.2	–	–	0.11	5.4	–	–	0.16
Yes	306	3.6	1.62	0.87, 3.03		6.9	1.30	0.82, 2.05	
Thinness									
No	6258	2.3	–	–	0.26	5.5	–	–	0.29
Yes	419	3.1	1.39	0.78, 2.47		4.3	0.77	0.47, 1.25	
Sickness within past 2 weeks									
No	4417	1.9	–	–	0.01	6.0	–	–	0.01
Yes	2260	3.1	1.69	1.23, 2.33		4.5	0.75	0.59, 0.94	

Comparison was made by Pearson's χ^2 ; OR (95% CI) was calculated as Mantel–Haenszel common OR estimate.

†Plasma vitamin A <0.70 $\mu\text{mol/l}$.

‡Plasma vitamin E <6.97 $\mu\text{mol/l}$.

$P=0.05$) and in those with a sickness within the past 2 weeks (OR = 1.51; 95% CI 1.09, 2.09; $P=0.01$). There was a trend towards significant association between VAD and underweight (OR = 1.81; 95% CI 0.92, 3.53; $P=0.08$) and living in the peri-urban region (OR = 1.36; 95% CI 0.96, 1.94; $P=0.08$). The independent predictors of VED were living in the peri-urban region (OR = 1.60; 95% CI 1.28, 2.01; $P<0.001$) and sickness within the past 2 weeks (OR = 0.75; 95% CI 0.60, 0.94; $P=0.01$).

Discussion

A serum retinol value only reflects the VA reserves when these have largely been depleted; concentrations under 0.70 $\mu\text{mol/l}$ reflect a moderate deficit, whereas a deficiency is considered to be severe when the level falls below 0.35 $\mu\text{mol/l}$ ⁽²⁶⁾. The recommended WHO cut-off point to estimate whether VAD in a community constitutes a public

health problem is a prevalence of 2% to <10% for mild, 10% to <20% for moderate and $\geq 20\%$ for severe deficiency. This is based on a serum retinol cut-off value of 0.70 $\mu\text{mol/l}$ ⁽²⁷⁾. VE status is not considered an indicator of the nutritional state. In addition, there is no consensus on the cut-off value to estimate whether VED is a public health problem. The prevalence of 2.3% for VAD and 5.4% for VED identifies the studied population as having mild public health problems of VAD and VED. No severe VA and VE deficiencies were observed. However, low plasma VA and VE concentrations were frequent (17% and 20.2%, respectively). The present study showed a higher prevalence of VAD in children with underweight or stunting, confirming the close relationship between chronic malnutrition and VAD, but not VED. Thinness that reflects altered current nutritional status was not associated either with VAD or with VED. One interesting finding of the present study is the higher frequency of VAD and VED in children who reside in peri-urban regions. This may be

explained by a low socio-economic rank of households living in these areas, as shown by higher rates of underweight, stunting and thinness. In favour of this statement, previous reports have shown that these regions are mostly inhabited by rural migrating poor communities with low income and high rates of short-term unemployment^(18,19). VAD was more frequent in 7-year-old children. In fact, being a 7-year-old in first grade of elementary school signifies educational backwardness that may be caused by unfavourable socio-economic conditions or recurrent sickness. Indeed, in the present study, these children showed the highest rates of underweight and stunting, and lived mainly in the disadvantaged peri-urban regions.

A high proportion of children (33.8%) presented with a sickness within the past 2 weeks, probably due to viral episodes related to the wintry season. VAD was more frequent in children with a sickness within the past 2 weeks. Low plasma retinol may be responsible for increased childhood morbidity due to impaired immune function. However, it might be a consequence of infection-induced changes in VA transport rather than of a true deficiency. It was shown that during the acute phase response, retinol concentration decreases transiently as a consequence of the decreased release of retinol-binding protein from the liver and increased urinary retinol loss^(28,29). Unlike VAD, VED was less frequent in children with a sickness within the past 2 weeks. This unexpected finding may be explained by the effect of inflammation on circulating lipids. It is well documented that acute inflammation increases plasma lipid levels⁽³⁰⁾. VE is transported in the plasma in the lipoproteins and, as a result, VE concentrations are related to the concentrations of lipids⁽³¹⁾. Inflammation-induced increase in circulating lipids and VE may explain lower VED prevalence in children with a sickness within the past 2 weeks. Thus, acute phase response decreases the reliability of use of plasma VA or VE as indicators of VA or VE status, because by status nutritionists typically mean liver stores⁽²⁴⁾. However, when excluding children with a sickness within the past 2 weeks, the prevalence of vitamin deficiencies did not vary significantly (1.9% for VAD and 6.0% for VED).

Our findings are based on a population survey involving a large number of children. But, unfortunately, biochemical determination was not achieved for over 10% of them. Data arose from multivariate analysis with adjustment on factors that may affect VA and VE status. However, as information was collected from 5- to 7-year-old children, key factors such as dietary intake or household income have not been investigated, which represents a significant limitation of the study. Some explanations of the low prevalence of VAD may be advanced. First, an improvement in the living standards of Tunisians results in ample food availability. Second, the expansion of vaccination and health programmes that target the child and mother results in better health for both. Third, diverse information dissemination on nutrition and feeding by the media made the Tunisian population

attach more importance to children's nutrition and health. The low prevalence of malnutrition in our children corroborates this assumption. The main reason for the rarity of VAD would be that the Tunisian diet includes a large variety of VA-rich foods. Indeed, pro-VA carotenoids are abundant in darkly coloured fruits and vegetables, which are largely consumed by Tunisians, even those of low socio-economic status^(19,32). Consumption of commercial food products fortified with VA and VE, such as milk and juice, may have enhanced vitamin status. However, these products are uncommon and costly, and as a consequence are consumed by few children⁽³²⁾. While VE-rich vegetable oils are commonly consumed by Tunisians, VE-rich foods, such as wheat germ oil, almonds, nuts, butter and oily fish, are less accessible^(19,30), which may explain why VED is more frequent than VAD in the children studied. A survey of demographic, socio-economic and health characteristics, and dietary intake could be undertaken in Tunisian children to further clarify the reasons for the low prevalence of VAD and VED. The present study reflects VA and VE status for the February/March period, a season during which darkly coloured fruits and vegetables are abundant. In addition, 5–7 years is not the age period in which VA or VE deficiency prevalence is highest. VAD and VED may be more frequent in younger children and during the hot season.

In conclusion, 2.3% of children showed plasma VA concentrations $<0.70 \mu\text{mol/l}$ and 5.4% showed VE concentrations $<6.97 \mu\text{mol/l}$, but none showed severe VA or VE deficiency. The rarity of VAD in our population could be related to the improvement of living standards and health-care, as well as the large accessibility to VA-rich foods. Taking into account that the Kasserine Governorate shows the lowest socio-economic, health and environmental indicators of the country, it is presumed that VAD and VED are not public health problems in Tunisia. However, more than one-fourth and nearly one-fifth of the children had low plasma concentrations of VE and VA, respectively. A reinforcement of the national strategy of health-care and nutritional education is needed, with a particular emphasis on the disadvantaged regions. Supplementation could not be considered necessary in Tunisian children. However, VA and VE food fortification may ensure that sufficient stores are built and that the infectious disease burden is impacted positively.

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analysis and interpretation of data. N.K., J.E.A, M.C.K. and P.T. revised the paper. All authors reviewed and approved the final version of the paper.

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