

## Comparison of serum concentration and dietary intake of $\alpha$ -tocopherol in a sample of urban and rural Costa Rican adolescents

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**SUMMARY.** Results from several studies have suggested that vitamin E intake could inhibit the progression of atherosclerotic lesions. Therefore, this study was designed to evaluate Costa Rican adolescents' serum  $\alpha$ -tocopherol levels and their correlation with Body Mass Index (BMI) and the dietary intake of vitamin E and saturated and polyunsaturated fat. Ninety-five healthy, non-smoking adolescents and non-vitamin supplement users (aged 13-18) from urban and rural areas in San José, Costa Rica, were included in this study. Serum levels of lipid adjusted  $\alpha$ -tocopherol were significantly higher in rural adolescents compared with urban youngsters ( $4.192 \mu\text{mol}/\text{mmol} \pm 0.831$  and  $3.486 \mu\text{mol}/\text{mmol} \pm 0.996$ , respectively). Likewise, reported mean daily 1000 kcal adjusted-vitamin E intake was higher in rural adolescents than in urban youngsters ( $9.2 \pm 3.7$  mg,  $16.0 \pm 8.7$  mg). An important correlation was observed between intake of energy from saturated fat and adjusted- $\alpha$ -tocopherol serum levels ( $r = 0.430$ ). Contrariwise, they correlated poorly with dietary  $\alpha$ -tocopherol ( $r = 0.273$ ), suggesting that serum is not a good biomarker of intake for  $\alpha$ -tocopherol. Additionally, our results showed a negative relationship between BMI and adjusted-serum vitamin E levels ( $\beta = -0.189$ ; CI 95%  $-0.153, -0.013$ ). The analysis of vitamin E intake showed that over 25% of adolescents have inadequate intake of the nutrient. This study suggests that nutritionists and pediatricians should encourage  $\alpha$ -tocopherol intake and monitor the food sources of the nutrient and the adolescents' body weight as part of the strategies aimed at developing a healthy lifestyle.

**Key words:**  $\alpha$ -tocopherol, vitamin E, dietary intake, serum levels, adolescents, Costa Rica.

**RESUMEN.** Comparación de la concentración sérica y del consumo de  $\alpha$ -tocopherol en una muestra de adolescentes urbanos y rurales de Costa Rica. Los resultados de diferentes estudios han sugerido que el consumo de vitamina E puede inhibir el progreso de las lesiones ateroscleróticas. Por tanto, este estudio fue diseñado para evaluar los niveles séricos de  $\alpha$ -tocoferol en adolescentes costarricenses y su relación con el Índice de Masa Corporal (IMC), el consumo de vitamina E, grasa saturada y poliinsaturada. Noventa y cinco adolescentes (13-18 años) saludables, no fumadores, no consumidores de suplementos vitamínicos y habitantes del área urbana y rural de San José-Costa Rica fueron incluidos en el estudio. Los niveles séricos de  $\alpha$ -tocoferol ajustados por lípidos fueron significativamente mayores en adolescentes rurales que en urbanos ( $4.192 \mu\text{mol}/\text{mmol} \pm 0.831$  y  $3.486 \mu\text{mol}/\text{mmol} \pm 0.996$  respectivamente). Así mismo, el consumo diario de vitamina E ajustado por 1000 Kcal fue mayor en los jóvenes rurales que en los urbanos ( $9.2 \pm 3.7$  mg,  $16.0 \pm 8.7$  mg). Los datos mostraron una importante correlación entre la ingesta de energía derivada de grasa saturada y los niveles séricos de  $\alpha$ -tocoferol ajustados por lípidos ( $r = 0.430$ ). Por el contrario se evidenció una débil correlación entre estos y la ingesta de vitamina E ( $r = 0.273$ ), sugiriendo que los niveles séricos no son buenos biomarcados de la ingesta del nutriente. Adicionalmente, se determinó una relación negativa entre los niveles séricos de vitamina E-ajustada por lípidos y el IMC ( $\beta = -0.189$ ; IC 95%  $-0.153, -0.013$ ). El análisis de la ingesta de vitamina E mostró que más del 25% de los adolescentes presenta un consumo inadecuado de este nutriente ( $< 2/3$  DRI). Nuestros datos sugieren que los nutricionistas y pediatras deben promover el consumo de  $\alpha$ -tocoferol y monitorear las fuentes alimentarias de este nutriente y el peso corporal de los adolescentes como parte de las estrategias orientadas al desarrollo de un estilo de vida saludable.

**Palabras clave:**  $\alpha$ -tocoferol, vitamina E, consumo dietético, niveles séricos, adolescentes, Costa Rica.

### INTRODUCTION

Diet is a significant issue in the prevention of primary and secondary Coronary Heart Disease (CHD) (1). Some dietary factors that protect against the development of CAD and other dietary factors are pathogenic, enhancing atherosclerosis. Several lines of scientific evidence have

shown that some of the saturated fatty acids, particularly myristic and lauric acids contribute to the causation of atherosclerosis (1-3), while  $\alpha$ -tocopherol may play a role in prevention (4-6). Vitamin E has been identified recently as a favorable modulator of several atherogenic processes at the molecular and cellular levels (7).

Vitamin E, mainly  $\alpha$ -tocopherol, is the major fat-soluble antioxidant present in the LDL particle. On average, 5-9 vitamin E molecules are carried by each LDL particle and protect it from oxidative modification (7-10). In addition,  $\alpha$ -tocopherol is incorporated into other components of the vascular system, including endothelial cells, smooth muscle cells, platelets and immune cells, and has been shown to modulate a variety of inflammatory processes that are involved in atherosclerosis (7,10,11). Vitamin E suppresses expression of adhesion molecules on endothelial cells and ligands on monocytes and reduces their adhesive interactions, which is an important early event in the initiation of fatty streak formation and atherogenesis (7,10,14).

A substantial body of evidence has indicated that dietary antioxidants might reduce the risk of atherosclerosis (7). In several studies in adults, high vitamin E intake has been shown to be associated with reduced CHD risk (15-16). Additionally, several studies have shown that subnormal  $\alpha$ -tocopherol plasma levels may contribute to the increased risk of atherosclerosis, mainly in obese subjects (17).

Since the process of atherosclerosis begins early in life (18,19), the evaluation of CHD risk factors previously identified in older subjects is essential during adolescence for stemming disease progression in later years. This is vital in Costa Rica where CHD represents the leading cause of death among adults (20).

This study was conducted to evaluate Costa Rican adolescents' serum  $\alpha$ -tocopherol levels and their correlation with Body Mass Index (BMI) and the dietary intake of vitamin E and saturated and polyunsaturated fat.

## SUBJECTS AND METHODS

Participants in this study were healthy, non-smoking and non-vitamin supplement using high school students. Adolescents were selected at random from 10 public high schools in urban and rural areas in the Province of San José. The high schools were selected with probability proportional to size from a list of all public high schools in the study area. Both parents and students gave their written consent to participate in the study. Ethical permission for the study was obtained from the Costa Rican Institute for Research and Education on Nutrition and Health's (Inciensa) Ethics Committee.

After 12-h fasting, blood samples were collected from the antecubital vein. Serum Vitamin E levels were measured by HPLC, based on the methodology recommended by Beiri et al. (21). Serum  $\alpha$ -tocopherol concentration was adjusted for serum lipids by dividing by the sum of serum cholesterol and triglyceride concentration, as has been suggested by Thurman et al. (22) and Horwitt et al. (23). Serum lipids were determined by enzymatic methods (Wiener), using an

automatic analyzer (ASCA; LSI Instruments).

Quartiles and deciles of serum adjusted-  $\alpha$ -tocopherol levels were calculated based on the entire adolescent population. Comparisons were made in the proportions of urban and rural adolescents at 10<sup>th</sup> and 25<sup>th</sup> percentiles of adjusted-  $\alpha$ -tocopherol levels.

Vitamin E intake was determined using prospective 3-d diet records including two weekdays and one weekend day. Series of three to six photographs of food usually eaten in Costa Rica were used for each adolescent to estimate portion size while keeping food records (24). Foods and three-dimensional food models were used to verify the size of some portions reported by adolescents. The Food Processor® for Windows version 6.0 (Esha Research, Salem, Oregon) was used to perform nutrient calculations from dietary data. Two thirds (or 66.7%) or less of the Dietary Reference Intake (DRI) for vitamin E (25) was used as the criterion for inadequate intake of this nutrient. Because the consumption of nutrients is at least partially dependent on total energy intake, and because the nutrient density of the diet is in this instance more relevant than actual gross intake, an adjustment for energy intake by computing nutrient intakes per 1000 kcal was performed.

Food groups were created to identify dietary sources of vitamin E. The contribution of total dietary vitamin E by each food group was determined using the following formula: (total grams of dietary vitamin E from all foods in a group)÷(total grams of dietary vitamin E from all foods).

Overweight was estimated using the Body Mass Index (weight / height<sup>2</sup>). Weight was measured without shoes and with heavy outer clothing removed. Height was measured with the student shoeless and facing away from the scale. Standing height was measured to the nearest 0.1 cm and weight was measured to the 0.1 kg. Independent duplicate measurements were obtained for height and weight, and the average of the two readings, required to be within  $\pm$  0.5 cm or 0.5 kg respectively, was used in data analysis.

Children with Body Mass Index (BMI)  $\geq$  85th percentile were considered to be overweight as suggested by the World Health Organization Expert Committee (26). In the absence of other data specifying optimum cut-off values for BMI in children; the BMI for age data for US children were used, as recommended by the WHO Expert Committee (26).

Data were examined with SPSS for Windows using the Mann-Whitney test to determine significant differences in lipids, dietary intake, and lipid adjusted- $\alpha$ -tocopherol levels between gender and geographic area. Differences in proportions were assessed with chi-square analysis. A Spearman correlation was calculated to determine associations between BMI, fat and vitamin E intake and adjusted-  $\alpha$ -tocopherol serum levels. Multiple regression analysis was used to develop a model with serum adjusted-

$\alpha$ -tocopherol as dependent variables. After examining univariate relationships between variables, multivariate stepwise models were initially used to identify which of the correlated variables provided the best model. Co-linearity was minimized by this approach, and correlation coefficients between independent variables included in the regression models did not exceed 0.3. A level of  $p < 0.05$  was considered significant.

## RESULTS

Of the 100 adolescents selected, those without the parents' written consent to participate in the study, or those with missing serum samples were excluded ( $n = 5$ ). The final

sample size of 95 adolescents consisted of 50 males ( $x \pm SD$  age:  $16.5 \pm 2.0$  y) and 45 females (aged  $15.9 \pm 1.7$  y). Forty-eight percent of the sample was randomly selected from the urban area and fifty-two percent from the rural area. All adolescents were from the same ethnic background (Mestizo).

The mean values for serum cholesterol and triglyceride levels from the adolescent population studied are presented in Table 1. There were no significant differences between urban and rural youngsters. Nevertheless, mean values for TC and TG for boys and girls were significantly different. TC mean value was 0.45 mmol/L lower in boys than girls ( $p=0.001$ ), for whom it was 4.14 mmol/L. Likewise TG levels were 0.15 mmol/L lower in boys than girls ( $p=0.001$ ), for whom it was 1.32 mmol/L.

TABLE 1  
Characteristics of the study population

Characteristic	Total population	Urban adolescents	Rural adolescents	<i>p value</i> <sup>1</sup>	Males	Females	<i>p value</i> <sup>1</sup>
N	95	46	49	—	50	45	—
Age (y)	$15.7 \pm 1.9$	$15.7 \pm 1.9$	$15.8 \pm 1.8$	0.804	$16.5 \pm 2.0$	$15.9 \pm 1.7$	0.574
Body Mass Index (BMI)	$20.7 \pm 3.1$	$20.6 \pm 2.8$	$20.8 \pm 3.4$	0.507	$20.5 \pm 3.7$	$21.1 \pm 2.2$	0.104
Overweight (%)	12.2	16.1	8.2	0.532	12.4	11.8	0.312
Total cholesterol (mmol/L)	$3.90 \pm 0.66$	$3.98 \pm 0.77$	$3.83 \pm 0.53$	0.628	$3.69 \pm 0.61$	$4.14 \pm 0.64$	0.001
Triglyceride (mmol/L)	$1.24 \pm 0.34$	$1.18 \pm 0.27$	$1.29 \pm 0.39$	0.229	$1.17 \pm 0.35$	$1.31 \pm 0.31$	0.001
Lipid adjusted - $\alpha$ -tocopherol ( $\mu\text{mol}/\text{mmol}$ )	$3.85 \pm 0.91$	$3.48 \pm 0.99$	$4.19 \pm 0.83$	0.000	$3.72 \pm 0.87$	$3.98 \pm 1.07$	0.412
Energy intake (Kcal)	$2119 \pm 729$	$2128 \pm 727$	$2112 \pm 731$	0.911	$2290 \pm 755$	$1930 \pm 648$	0.005
Vitamin E intake (mg $\alpha$ -TE)	$27.6 \pm 15$	$20.3 \pm 11.9$	$33.3 \pm 19$	0.022	$28.3 \pm 15.3$	$27.0 \pm 12.5$	0.889
Vitamin E intake/1000 Kcal (mg $\alpha$ -TE)	$13.0 \pm 6.2$	$9.2 \pm 3.7$	$16.0 \pm 8.7$	0.005	$11.7 \pm 8.8$	$14.5 \pm 7.1$	0.268
Energy from saturated fat (%)	$13.2 \pm 5.1$	$11.2 \pm 4.2$	$15.3 \pm 6.2$	0.001	$13.4 \pm 4.2$	$12.6 \pm 5.9$	0.908
Energy from polyunsaturated fat (%)	$5.9 \pm 2.7$	$6.6 \pm 2.8$	$5.4 \pm 2.6$	0.026	$5.5 \pm 2.0$	$6.3 \pm 3.2$	0.758

<sup>1</sup>Tested with Mann-Whitney test

Lipid-adjusted serum vitamin E averaged  $3.85 \pm 0.976$  mol/mmol (Table 1). Levels of serum adjusted  $\alpha$ -tocopherol were significantly higher in rural adolescents compared with urban youngsters. There were no significant differences between boys and girls. Percentiles of adjusted-serum  $\alpha$ -tocopherol levels are shown in Table 2. Adjusted serum  $\alpha$ -tocopherol levels were below the lowest quartile ( $\leq 3.158$   $\mu\text{mol}/\text{mmol}$ ) in 43.5% of urban adolescents compared with only 4.1% of rural adolescents ( $p < 0.001$ ). In addition,  $\alpha$ -tocopherol levels were below the lowest decile ( $\leq 2.791$   $\mu\text{mol}/\text{mmol}$ ) in 17.4% of urban youngsters compared with 2.0% of rural adolescents ( $p < 0.001$ ).

Table 1 shows the reported mean daily vitamin E intake. Mean intake was higher in rural adolescents than in urban youngsters ( $33.3$  mg  $\pm 19.2$  and  $20.3$  mg  $\pm 11.9$  respectively,  $p = 0.022$ ). This pattern was similar even when vitamin E intake was adjusted per 1000 Kcal. Likewise, although total energy intake was higher in males than in females ( $p = 0.005$ ),

no differences between the boys' and girls' energy-adjusted vitamin E intake was found ( $p = 0.268$ ).

TABLE 2  
Percentiles of adjusted- $\alpha$ -tocopherol levels for a sample of Costa Rican adolescents

Percentiles	Total population	Urban adolescents	Rural adolescents	Males	Females
10	2.787	2.407	3.414	2.499	2.922
20	3.031	2.825	3.499	2.945	3.087
25	3.158	2.904	3.599	3.214	3.155
30	3.414	2.936	3.724	3.437	3.250
40	3.494	3.065	3.859	3.494	3.489
50	3.650	3.342	4.056	3.582	3.780
60	3.861	3.476	4.360	3.771	4.229
70	4.358	3.558	4.496	3.998	4.464
75	4.440	3.614	4.578	4.363	4.578
80	4.606	4.110	4.619	4.476	4.691
90	5.191	5.143	5.328	5.175	5.477

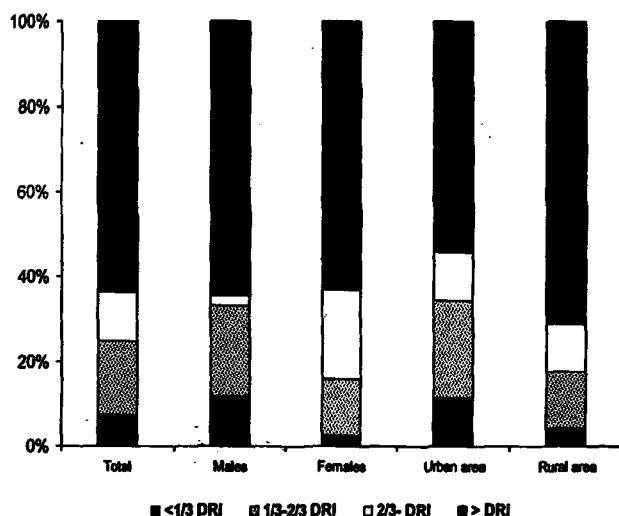
Rural adolescents reported a significantly greater energy intake from saturated fat than urban youngsters ( $p=0.001$ ), who reported a higher intake of energy from polyunsaturated fatty acids ( $p=0.026$ ) (Table 1).

Palm shortening contributed to 76% of vitamin E intake in rural areas and 35% in urban areas. Soy-bean oil contributed 15% of  $\alpha$ -tocopherol in urban areas and only 3% in rural areas. Margarine was the second contributor of vitamin E. It contributed more  $\alpha$ -tocopherol in urban than in rural areas (35% and 8% respectively,  $p < 0.001$ ).

Over 25% of the adolescents did not meet sixty-six percent of the DRI for vitamin E (Figure 1). The proportion of urban adolescents and males who did not meet 2/3 DRI for vitamin E tended to be higher (although not significantly) than the proportion of rural youngsters and females.

FIGURE 1

Percentage of Costa Rican adolescents meeting the Dietary Reference Intake (DRI) for vitamin E



The mean BMI was  $20.7 \pm 3.1$  (Table 1). There were no significant differences between urban and rural adolescents. The prevalence of overweight was 12.2%. Overweight prevalence tends to be higher, although not significantly, in urban adolescents than in rural youngsters. No differences were found between genders.

Spearman correlation coefficients between dietary variables, BMI and lipid-adjusted  $\alpha$ -tocopherol are shown in Table 3. The strongest correlation was observed between the intake of energy from saturated fat and adjusted- $\alpha$ -tocopherol serum levels ( $r = 0.430$ ). Contrariwise, serum adjusted  $\alpha$ -tocopherol correlated poorly with dietary intake of  $\alpha$ -tocopherol ( $r = 0.273$ ), energy from polyunsaturated fat ( $r = -0.283$ ) and BMI ( $r = -0.209$ ). Dietary vitamin E correlated strongly with energy from saturated fat ( $r = 0.813$ ) and negatively with energy from polyunsaturated fat ( $r = -0.365$ ).

TABLE 3

Spearman correlation coefficients (and  $p$  value) between dietary variables, Body Mass Index and lipid adjusted- $\alpha$ -tocopherol serum levels

Variables	Lipid adjusted $\alpha$ -tocopherol	Vitamin E intake
Vitamin E intake	0.273 (0.016)	
Energy from saturated fat intake	0.430 (0.000)	0.813 (0.000)
Energy from polyunsaturated fat intake	-0.283 (0.013)	-0.365 (0.001)
Body Mass Index	-0.209 (0.045)	0.022 (0.849)

A linear regression model with adjusted-serum vitamin E levels as dependent variables is presented in Table 4. This regression model explained about 27% of the variance in Costa Rican adolescents' serum vitamin E levels. After adjustment for age, a negative relationship between geographic area (95% CI -1.104, -0.264), gender (95% CI -0.819, -0.026), BMI (95% CI -0.153, -0.013) and adjusted-serum vitamin E levels was found. Dietary variables were not important predictors for adjusted-  $\alpha$ -tocopherol serum levels.

TABLE 4

Regression models with lipid adjusted- $\alpha$ -tocopherol levels as dependent variable

Independent variables	Estimated coefficient	95% CI
<b>Adjusted-<math>\alpha</math>-tocopherol levels</b>		
Age	-0.013	-0.105, 0.130
Gender <sup>1</sup>	-0.422	-0.819, -0.026
Area <sup>2</sup>	0.684	-1.104, -0.264
Body Mass Index	-0.189	-0.153, -0.013
Energy from saturated fat	0.061	-0.002, 0.015
Energy from polyunsaturated fat	0.011	-0.067, 0.090
$R^2 = 0.274$		

<sup>1</sup> Male=1, Female = 0, <sup>2</sup> Urban=1, Rural=0

## DISCUSSION

This study demonstrates that the levels of serum  $\alpha$ -tocopherol are significantly lower in urban Costa Rican adolescents compared with those adolescents living in rural areas. The adjusted-vitamin E serum levels in rural adolescents observed were similar to those reported by Decsi et al. for non-obese adolescents (17). An important prevalence of adjusted-serum vitamin E levels lower than  $2.7 \mu\text{mol}/\text{mmol}$  was found in urban youngsters. This requires more study, as levels of adjusted-vitamin E lower than  $2.67 \mu\text{mol}/\text{mmol}$  have been associated with higher rates of plasma lipid oxidation compared with levels higher than  $3.39 \mu\text{mol}/\text{mmol}$  (27).

We found a small correlation between dietary vitamin E and adjusted- serum  $\alpha$ -tocopherol ( $r = 0.274$ ), suggesting that serum is not a good biomarker of intake for  $\alpha$ -tocopherol. This finding is consistent with other studies including subjects who were not taking vitamin supplements (28-30). This smaller correlation may be due, at least in part, to genetic differences in absorption and metabolism. Polymorphisms in the  $\alpha$ -TTP gene have been associated with low plasma concentrations of  $\alpha$ -tocopherol in subjects with normal intake of vitamin (31). Mutations in this or other genes may, therefore, be important determinants of the serum response to dietary  $\alpha$ -tocopherol.

However, we found a strong correlation ( $r=0.430$ ) between intake of energy from saturated fat and adjusted-serum  $\alpha$ -tocopherol, specially in rural adolescents. This suggest an important association between serum  $\alpha$ -tocopherol levels and palm shortening intake, because this food is the primary contributor of saturated fat in the Costa Rican adolescents' diet (32). This observation is not compatible with observations by El-Sohemy et al. in a study with Costa Rican adults (30). They no found association between plasma  $\alpha$ -tocopherol levels and the type of fat (soy-bean oil, corn oil or palm shortening) used for cooking and frying at home. The differences between both studies reinforce the evidence that serum  $\alpha$ -tocopherol, unlike  $\gamma$ -tocopherol, does not adequately reflect intake from food sources (33).

Given that palm shortening contains 6 more  $\alpha$ -tocopherol/100 g than soy-bean oil (21.6mg and 16 mg, respectively) (34), the higher palm shortening intake in rural areas explains our results. Unfortunately, palm shortening is an important contributor of atherogenic palmitic acid (C16: 0) (35). Although palmitic acid exerts a lower effect on the plasma lipids than miristic acid (C14: 0) and lauric acid (C12: 0) (35), the reduced intake of this saturated fatty acid has resulted in a reduction in plasma LDL-cholesterol levels in well-controlled dietary studies (36).

Recent results from subgroup analysis of the Cholesterol Lowering Atherosclerosis study (CLAS) and other studies suggest that high vitamin E intake could inhibit lesion progression (7,37,38). Therefore, it is wise to ensure an adequate intake of  $\alpha$ -tocopherol beginning in adolescence because it could have important public health benefits. It appears important, since according to current vitamin E dietary reference intake (15 mg/d), over 25% of adolescents showed an inadequate intake of this antioxidant. However; the  $\alpha$ -tocopherol food contributors should receive special attention in order to reduce the atherogenic characteristics in the adolescents' diet. The goal of adequate vitamin E intake should be achieved by minimizing saturated vegetable fat intake and replacing it with unsaturated vegetable fat as has been suggested by the Hohenheimer Consensus Meeting (39). In addition, although diet alone does not provide the levels

of vitamin E intake associated with the lowest risk for cardiovascular disease, the absence of efficacy and safety data from randomized trials precludes vitamin E supplementation (36).

Correcting suboptimal  $\alpha$ -tocopherol intake is a true preventive measure for CHD development in healthy people (4); however an increase in the intake of  $\alpha$ -tocopherol cannot compensate for the effect of an atherogenic diet or excess weight. Bieri et al. (40) have suggested that sequestration of  $\alpha$ -tocopherol in adipose tissue of obese subjects may limit its availability to other tissues, resulting in lower adjusted  $\alpha$ -tocopherol serum levels. This relationship was confirmed in this study; for each unit of increase on the BMI, the adjusted-vitamin E levels will diminish 0.189 $\mu$ mol/mmol.

Low adjusted-serum  $\alpha$ -tocopherol levels may contribute to the increased risk of cardiovascular disease associated with obesity. Strauss (41) has suggested that modestly decreased levels of  $\alpha$ -tocopherol in obese adolescents may be of sufficient magnitude to affect lipid oxidation. This is worrisome, as 12% of the adolescents studied presented overweight. In addition, 36% of these adolescents presented levels of total cholesterol higher than 4.4mmol/L (data not shown). High concentration of candidate target molecules for lipid peroxidation combined with reduced availability of the most important lipid-soluble antioxidant may be one of the many factors predisposing overweight adolescents to a high risk for the development of atherosclerosis later in life (17).

Our results suggest that vitamin E intake should be promoted in adolescents. However, it should be encouraged as a combination of strategies aimed at developing a healthy lifestyle, with particular emphasis on reducing the saturated fat intake and the incidence of obesity to reduce health risks in later life.

## REFERENCES

1. Berenson G, Srinivasan S, Nicklas T. Atherosclerosis: A nutritional disease of childhood. *Am J Cardiol* 1998; 82: 22T-29T.
2. Hegsted DM, Ausman L, Johnson J, Dallal G. Dietary fat and serum lipids: an evaluation of the experimental data. *Am J Clin Nutr* 1993; 57: 875-883
3. Hayes KC, Khosla P. Dietary fatty acid thresholds and cholesterolemia. *Faseb J* 1992;6: 2600-2607.
4. Tribbe D. Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin C, vitamin E and  $\beta$ -carotene. A statement for healthcare professionals from the American Heart Association. *Circulation* 1999;99: 591-595.
5. Krebt P, Reunanen A, Jarvinen R, Seppanen R, Heliovaara M, Aromaa A. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1994; 139: 1180-1189.

6. Machlin L. Critical assessment of the epidemiological data concerning the impact of antioxidant nutrients on cancer and cardiovascular disease. *Food Sci Nutr* 1995; 35: 41-50.
7. Meydani M. Vitamin E and atherosclerosis: Beyond prevention of LDL oxidation. *J Nutr* 2001; 131: 366S-368S.
8. Evstigneeva RP, Volkov IM, Chudinova VV. Vitamin E as a universal antioxidant and stabilizer of biological membranes. *Membr Cell Biol* 1998; 12: 151-172.
9. Sakuma N, Yosikawa M, Hibino T, Okada M, Jinno Y, Tamai N, et al. Alpha-tocopherol protects the peroxidative modification of LDL to be recognized by LDL receptors. *J Nutr Sci Vitaminol Tokyo* 1998; 44: 697-703.
10. Chan AC. Vitamin E and Atherosclerosis. *J Nutr* 1998; 128: 1593-1596.
11. Azzi A, Boscoboinik D, Clement S, Marilley D, Ozer NK, Ricciarelli R, et al. Alpha-tocopherol as a modulator of smooth muscle cell proliferation. *Prostaglandins Leukot Essent Fatty Acids* 1997; 57: 507-514.
12. Devaraj S, Jialal I. The effects of alpha tocopherol on critical cells in atherogenesis. *Curr Opin Lipidol* 1998; 9: 11-15.
13. Liao J. Endothelium and acute coronary syndromes. *Clin Chem* 1998; 44: 1799-1808.
14. Azen SP, Qian D, Mack WJ, Sevanian A, Selzer RH, Liu CR, et al. Effect of supplementary antioxidant vitamin intake on carotid arterial wall intima-media thickness in a controlled clinical trial of cholesterol lowering. *Circulation* 1996; 94: 2369-2372.
15. Stampher MJ, Hennekens CH, Manson JE, Colditz GA, Rosner M, Willet WC. Vitamin E consumption and the risk of coronary heart disease in women. *N Engl J Med* 1993; 328: 1444-1449.
16. Rimm EB, Stampher MJ, Ascherio A, Giovannucci E, Colditz GA, Willet WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; 328: 1450-1456.
17. Decsi T, Molnár D, Koletzko B. Reduced plasma concentrations of alpha-tocopherol and beta-carotene in obese boys. *J Pediatr* 1997; 130: 653-655.
18. McGill H, McMahan A, PDAY Research Group. Determinants of atherosclerosis in the young. *Am J Cardiol* 1998; 82: 30T-36T.
19. Berenson G, Wattigney W, Bao W, Srinivasan S, Radhakrishnamurthy B. Rationale to study the early natural history of heart disease: The Bogalusa Heart Study. *Am J Med Sci* 1995; 310: S22-S28.
20. Morice A. Análisis de la situación de las enfermedades crónicas no transmisibles en Costa Rica, Tres Ríos: INCIENSA, 1998.
21. Beiri JG, Tolliver JT, Catigman G. Simultaneous determination of tocopherol and retinol in plasma or red cells by High Pressure Liquid Chromatography. *Am J Clin Nutr* 1979; 32: 2143-2149.
22. Horwitt MK, Harvey CC, Dahm CH, Scarcy MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann NY Acad Sci* 1972; 203: 203-236.
23. Thurman DI, Davies JA, Crump BJ, Situnajake RD, Davis M. The use of different lipids to express serum tocopherol: lipid ratio for measurement of vitamin E status. *Ann Clin Biochem* 1986; 23: 514-520.
24. Araúz AG. Método de registro de alimentos de tres días. In: Madrigal H, Martínez H, eds. *Manual de encuestas de dieta*. México: Instituto Nacional de Salud Pública 1996; 83-98.
25. Monsen E. Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium and carotenoids. *J Am Diet Assoc* 2000; 100: 637-640.
26. World Health Organization. *Physical status: the use and interpretation of anthropometry*. WHO technical report series. 854. Switzerland: WHO; 1995.
27. Haffner SM, Miettinen H, Stern MP, Agil A, Jialal Y. Plasma oxidizability in Mexican-Americans and Non-Hispanic whites. *Metabolism* 1996; 45: 876-81.
28. Kardinaal AFM, van't Veer P, Bramts HAM, van den Berg H, van Schoonhoven J, Hermus RJJ. Relation between antioxidant vitamins in adipose tissue, plasma, and diet. *Am J Epidemiol* 1995; 141: 440-450.
29. Ascherio A, Stampher MJ, Colditz GA, Rimm EB, Litin L, Willet WC. Correlations of vitamin A and E intakes with the plasma concentration of carotenoids and tocopherols among American men and women. *J Nutr* 1992; 122: 1792-1801.
30. Eh-Soheemy A, Baylin A, Ascherio A, Kabagambe E, Spiegelman D, Campos H. Population-based study of  $\alpha$ - and  $\gamma$ -tocopherol in plasma and adipose tissue as a biomarker of intake in Costa Rican adults. *Am J Clin Nutr* 2001; 74: 356-363.
31. Cavalier L, Ouahchi K, Kayden HJ, DiDonato S, Reutenauer L, Mandel JL, et al. Ataxia with isolated vitamin E deficiency: heterogeneity of mutations and phenotypic variability in a large number of families. *Am J Hum Genet* 1998; 62: 301-310.
32. Monge-Rojas R. Dietary intake as a cardiovascular risk factor in Costa Rican adolescents. *J Adolesc Health* 2000; 28: 228-337.
33. Dimitrov NV, Meyer C, Gilliland D, Ruppenthal M, Chenoweth W, Malone W. Plasma tocopherol concentration in response to supplemental vitamin E. *Am J Clin Nutr* 1991; 53: 723-729.
34. Cotrell R. Nutritional aspects of Palm oil. *Am J Clin Nutr* 1991; 53: 989S-1009S.
35. Hayes KC, Pronczuk A, Lindsey S, Diersen-Schade D. Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in non human primates. *Am J Clin Nutr* 1991; 53: 491-498.
36. Kraus R, Eckel R, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, et al. AHA dietary guidelines for healthy American adults. Revision 2000: A statement for health professionals from the Nutrition Committee American Heart Association. *Circulation* 2000; 102: 2284-2299.
37. Azen SP, Qian D, Mack WJ, Sevanian A, Selzer RH, Lui CR, et al. Effect of supplementary antioxidant vitamin intake on carotid arterial wall intima-media thickness in a controlled clinical trial of cholesterol lowering. *Circulation* 1996; 94: 2369-2372.
38. Hodis HN, Mack WJ, LaBree L, Cashin-Hemphill L, Sevanian A, Johnson R, et al. Serial coronary angiographic evidence

- that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. *JAMA* 1995; 273: 1849-1854.
39. Biesalski HK, Bohler H, Esterbauer H, Furst P, Hundsdorfer G, Kasper H, et al. Antioxidant vitamins in prevention. *J Clin Nutr* 1997; 16: 151-155.
40. Bieri JG, Poukla E, R. Effect of plasma lipids levels and obesity on tissue stores of a-tocopherol. *Proc Soc Exp Biol Med* 1975; 149: 500-2.
41. Strauss R. Comparison of serum concentrations of  $\alpha$ -tocopherol and  $\beta$ -carotene in a cross-sectional sample of obese and nonobese children (NHANES III). *J Pediatr* 1999, 134:160-5.

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