Original Communication

Antioxidant Vitamin Status (A, E, C, and Beta-Carotene) in European Adolescents – The HELENA Study

Christina Breidenassel[2], Jara Valtuena[2,8], Marcela González-Gross[1,2,3], Jasmin Benser[1], Andre Spinneker[1], Luis A. Moreno[4], Stefaan de Henauw[5], Kurt Widhalm[6], Denes Molnar[7], Guiseppe Maiani[8], and Peter Stehle[1] on behalf of the HELENA Study group*

[1]Institut für Ernährungs- und Lebensmittelwissenschaften – Humanernährung, Rheinische Friedrich-Wilhelms Universität Bonn, Bonn, Germany
[2]ImFINE Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences (INEF) Universidad Politécnica de Madrid, Madrid, Spain
[3]Grupo EFFECTS 262, Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Granada, Spain
[4]GENUD (Growth, Exercise, Nutrition and Development) Research Group, EU Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain
[5]Department of Public Health, Ghent University, Ghent, Belgium
[6]Medical University of Vienna, Vienna, Austria
[7]Pécsi Tudományegyetem (University of Pécs), Pécs, Hungary
[8]Human Nutrition Unit, INRAN, Rome, Italy

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Abstract: *Background:* An adequate nutritional status of antioxidant vitamins (vitamins A, C, E) and β-carotene is essential especially during childhood and adolescence, because of their important roles in cell growth and development. Currently, there are no physiological reference values for blood concentration of these vitamins and β-carotene in apparently healthy European adolescents. The aim of the current study was to obtain reliable and comparable data of antioxidant vitamins and β-carotene in a cross-sectional study, within HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence), which was conducted in a representative sample of adolescents from ten European cities. *Material and Methods:* From a subsample of 1,054 adolescents (males= 501) of the HELENA Cross Sectional Study with an age range of 12.5 to 17.49 years, fasting blood samples were taken and analyzed for vitamins A, E, C, and β-carotene status. As specific ref-

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erence values for adolescents are missing, percentile distribution by age and sex is given. *Results:* Mean concentrations were the following: Retinol: 356.4 ± 107.9 cm/mL; α-tocopherol: 9.9 ± 2.1 μg/mL; vitamin C: 10.3 ± 3.3 mg/L; and β-carotene: 245.6 ± 169.6 cm/mL. Females showed higher α-tocopherol and vitamin C values compared with males and 17-year-old boys had higher retinol levels than the same-aged girls ($p = 0.018$). Retinol serum concentrations increased significantly according to age in both gender, but girls had also significantly increasing β-carotene levels by age. *Conclusions:* For the first time, concentrations of antioxidant vitamins and pro-vitamin β-carotene have been obtained in a representative sample of apparently healthy European adolescents. These data can contribute to the establishment of reference ranges in adolescents.

Key words: α-tocopherol, vitamin C, retinol, beta-carotene, adolescents, Europe

Introduction

Research in recent years has highlighted the physiological importance of antioxidant vitamins A, C, E and the pro-vitamin β-carotene, and has shown that their biological activities are much broader than expected [1]. In fact, they are specifically involved in multiple cellular and tissue processes [2] that go beyond the antioxidant function [3]. Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction [4]. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and provitamin A carotenoids, i. e. β-carotene [5]. Beta-carotene is receiving increasing interest because of its high antioxidant power [6].Vitamin C (ascorbic acid) is necessary for an optimal immune function and is an essential cofactor for hydroxylation of proline and lysine, collagen synthesis, and connective tissue integrity [6–8]. Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system and is exclusively obtained from the diet. As a chain-reaction breaking antioxidant vitamin E prevents the propagation of lipid-peroxidation, especially of polyunsaturated fatty acids (PUFAs) and other components of cell membranes and low-density lipoproteins (LDL) from oxidation by free radicals [5, 6].

There is increasing evidence linking antioxidant vitamins and β-carotene with chronic diseases like cardiovascular and cerebrovascular disease, cancer, and diabetes in adulthood [2, 9, 10]. High oxidative stress and free radical levels are involved in the development of cardiovascular disease and other undesirable metabolic situations in adulthood [3, 11]. But as research has also shown in the last decade, risk factors are already established during childhood and adolescence [12]. In addition to the increasing needs in nutrients and energy for an adequate growth and development [9, 13], having an adequate vitamin status could have an additional beneficial health effect during adolescence. But little is known about the reference blood levels of these vitamins during adolescence [14]. There is a general consensus in the literature that these values are missing for the adolescent population [2, 15–17]. As we reviewed recently [18], studies performed on European adolescents specifically dealing with the above-mentioned vitamins and β-carotene are scarce, and in several cases these studies were performed more than 10 or 15 years ago [14, 19–21]. One of the main aims of the HELENA-Study (Healthy Lifestyle in Europe by Nutrition in Adolescence) was to provide, for the first time, reliable data about micronutrient status in European adolescents [4, 6, 22].

The main objective of the current study is to obtain reliable and comparable data of a representative sample of European adolescents, concerning the assessment of vitamin A, C, E and β-carotene status in adolescents, to analyze vitamin plasma concentrations by sex and age, and to contribute to percentile distribution for clinical use as reference values which are missing for the adolescent population [23, 24].

Material and Methods

Study design and subject recruitment

A European multicenter cross-sectional study (CSS) was performed in adolescents aged 12.5–17.49 years (HELENA, www.helenastudy.com). The general methodology has been described in detail elsewhere [24, 25]. Briefly, 3,000 apparently healthy adolescents were recruited in ten cities of more than 100,000 habitants across Europe, selected on the basis of two criteria: (a) regional distribution (Northern, Southern, Western, Eastern, Central Europe), and (b) presence of an active research group assuring sufficient expertise and resources to successfully perform this epidemiological study. Study centers were in Stockholm (Sweden), Athens and Heraklion (Greece), Rome (Italy), Zaragoza (Spain), Pecs (Hungary), Ghent (Belgium), Lille (France), Dortmund (Germany), and Vienna (Austria). Subjects were recruited by school-based, multiple-step, stratified random, and cluster sampling selections. Exclusion criteria were limited to subjects who were unable to speak the local language and participating simultaneously in another clinical trial. Blood sampling was performed in representative subsamples of 1,089 adolescents between October 2006 and October 2007.

All protocols and informed consents for this study were reviewed and approved by an Ethics Review Committee in each country according to the Declaration of Helsinki 1964 (revision of Edinburgh 2000) and the International Conferences on Harmonization for Good Clinical Practice [26]. Quality control was assured throughout the whole project as described by Beghin *et al.* [27]. Prior to the start of the HELENA-CSS, all methods had been tested in a pilot study to assure adequate sampling and to optimize transport logistics and analytics. These results have been described in detail previously [28].

Anthropometric measurements

Various anthropometric measures were performed using validated methods and standardized approaches as described previously [29]. Briefly, body weight was measured after blood sampling in underwear and without shoes using an electronic scale (Type SECA 861) to the nearest 0.1 kg, and height was measured barefoot in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm. The Body Mass Index (BMI) of the adolescents was calculated from their measured height and weight [BMI = weight divided by height squared (kg/m^2)].

Maturity was assessed by means of Tanner stage [30]. If maturity revealed different results when assessing gonads/breasts and pubic hair, the higher grade was chosen. Graduation into five grades of biological maturity ranging from no development (Tanner stage I) to complete development (Tanner stage V) was made to assess the influence of biological maturity on vitamin status [30].

Sample pre-treatment and transport

The blood sampling procedure and sample logistics within the HELENA-CSS have been described in detail [28]. Briefly, fasting blood sampling generally took place between 8 and10 a.m. Approximately 30 mL of blood were collected from an antecubital vein in serum, lithium heparin, and EDTA tubes (Sarstedt AG & Co., Nümbrecht, Germany) for assessing the different biomarkers and blood parameters, including vitamin C, α-tocopherol, retinol, and β-carotene. In order to coordinate the field work between the centers and the central laboratory at the University of Bonn (IEL), a blood-sampling calendar was developed. Blood-sampling date was dependent from local field work planning, agreement of the school and availability and capacity of the lab at IEL.

For vitamin C measurements the heparin tubes were put immediately on ice and were centrifuged within 30 minutes (3,500 × g, 15 minutes). For stabilization heparin plasma was precipitated with a 6 % (w/w) perchloric acid solution spiked with metaphosphoric acid (1:1). Serum samples for retinol vitamin E and β-carotene analysis were clotted at room temperature for at least 30 min and then centrifuged (3500 rpm, for 15 min) and the supernatants were transported at room temperature to the central laboratory and stored at –80 °C until analysis. For α-tocopherol, retinol, and β-carotene analysis EDTA plasma was used, therefore the EDTA tubes were sent at room temperature to the central laboratory and centrifuged 15 minutes at 3,500 × g at 4 °C and the supernatants were stored at –80 °C until analysis. For all samples freeze-thaw cycles were avoided until analysis.

Biochemical analyses

Retinol, α-tocopherol, and β-carotene were analyzed in serum using RP-HPLC (Sykam Gilching Germany) using UV-detection (UV-ViS 205, Merck Darmstadt, Germany). The separation was carried out on a LiChrosper Si 100 RP-18 column (125 mm × 4 mm, 5 μm Merck, Darmstadt) with an isocratic mobile phase consisted of hexane/isopropanol (98/2; wt/wt). The coefficient of variation (CV) of the method was 2.9 %.

Plasma vitamin C was analyzed in plasma by reverse phase-HPLC (Sykam Gilching Germany) using UV-detection (UV-ViS 205, Merck Darmstadt, Germany). Separation was carried out on a LiChrosper Si 100 RP-18 column (125 mm x 4 mm, 5 μm Merck, Darmstadt) and an isocratic mobile phase. The mobile phase consisted of 0.045 mmol/L sodium dihydrogenphosphate1-hydrate buffer adjusted at pH 2.0 with orthophosporic acid (14 %, w/w). The CV of method was: 1.7 %.

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (version 17.0, SPSS Inc., Chicago, IL, U.S.A.). To assess the influence of age, adolescents were stratified into 4 gender-specific age groups ranging from 12.5–13.99, 14–14.99, 15 15.99, and 16–17.49 years, respectively. All the analyses conducted on the HELENA data are adjusted by a weighing factor to balance the sample according to the theoretical sample in order to ensure true representation of each of the stratified groups.

Descriptive statistics were performed and values are shown as mean ± standard deviation (SD) and percentiles. The differences between sex and age groups were analyzed using one-way ANOVA. The Bonferroni *post-hoc test* was used for sub-group analysis.

To provide percentile values for European adolescents, we analyzed vitamin A, C, α-tocopherol, and β-carotene data by maximum penalized likelihood using the LMS statistical method for boys and girls separately [31, 32]. We derived smoothed centile charts using the LMS method. This estimates the measurement centiles in terms of three age–sex-specific cubic spline curves: the L curve (Box-Cox power to remove skewness), M curve (median), and S curve (coefficient of variation). For the construction of the percentile curves, data were imported into the LmsChartMaker software (V. 2.3; by Tim Cole and Huiqi Pan) and the L, M, and S curves estimated.

Results

Subjects' characteristics and mean retinol, β-carotene, α-tocopherol, and vitamin C concentrations for the total sample and by sex are shown on Table I. Girls had significantly higher vitamin C ($p = 0.003$) and α–tocopherol ($p \leq 0.001$) plasma values than boys.

Retinol concentrations increased with age in both genders ($p \leq 0.001$), but only girls had higher β-carotene levels as they increased in age ($p = 0.033$). Alpha-tocopherol concentrations were significantly higher in girls aged 16 years compared to 13-year-old girls ($p = 0.006$) (data not shown).

Comparing results by gender and age groups, significant differences were observed for retinol, α-tocopherol, and vitamin C concentrations. Females showed in general significantly higher α-tocopherol values compared with males ($p < 0.001$, data not shown) and higher vitamin C levels in the age group 14.0–14.99 years ($p = 0.019$). Males had a higher retinol status in the oldest age group compared to girls ($p = 0.018$). Percentile distribution for each vitamin presented by age groups is shown on Tables II–VII.

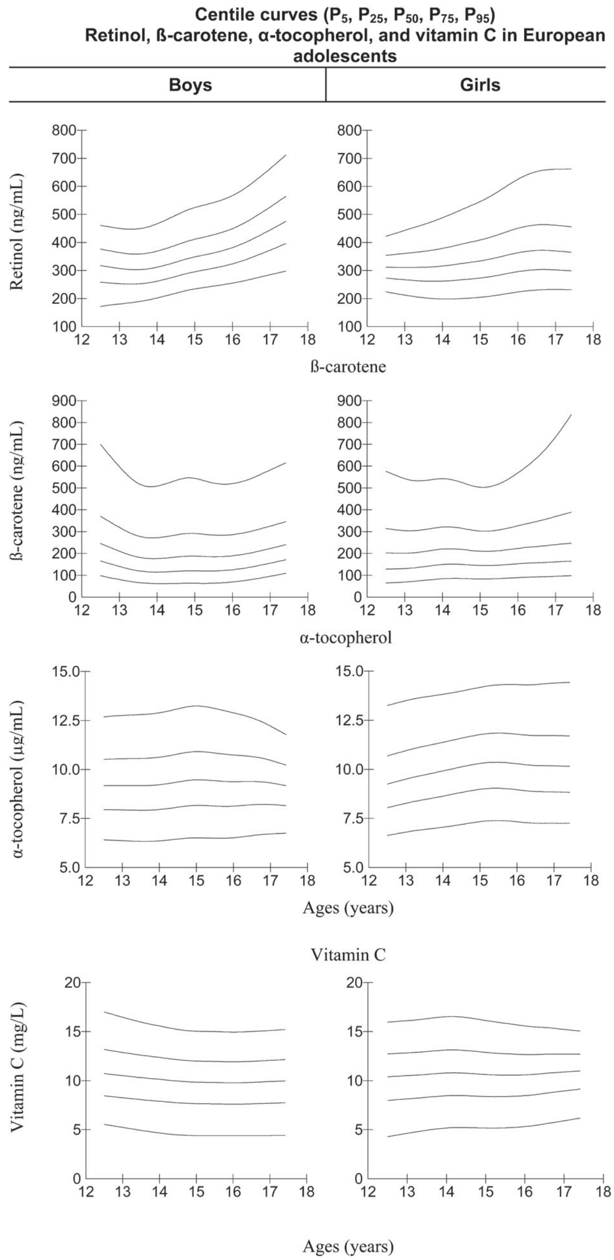

Figure 1: Smoothed (LMS method) centile curves (from the bottom to the top: P5. P25. P50. P75. P95) of retinol, β-carotene, α-tocopherol, and vitamin C in apparently healthy European adolescents.

Table I: Characteristics of the HELENA study population of 12.5–17.49 y-old adolescents by sex[1]

	total	**male**	**female**	**p**
	1053	501	553	
Age (y)	14.9 ± 1.2	14.9 ± 1.3	14.9 ± 1.2	NS
Height (cm)	165.9 ± 9.4	170.3 ± 9.8	161.9 ± 7.0	0.001
Weight (kg)	59.3 ± 12.7	62.6 ± 14.2	56.2 ± 10.2	0.001
BMI (kg/m^2)	21.4 ± 3.7	21.5 ± 4.0	21.4 ± 3.4	NS
Tanner stage (%) Stages I/II/III/IV/V	1/6/19/44/32	1/7/18/43/31	0/4/20/44/32	NS
Retinol (ng/mL)[1]	356.4 ± 107.9	362.6 ± 104.3	350.7 ± 110.8	NS
β-carotene (ng/mL)[2]	245.6 ± 169.6	236.5 ± 180.3	254.0 ± 159.1	NS
α-Tocopherol (µg/mL)[1]	9.9 ± 2.1	9.5 ± 2.0	10.3 ± 2.1	0.001
Vitamin C (mg/L)	10.3 ± 3.3	10.0 ± 3.3	10.6 ± 3.3	0.003

[1]n = 933 (male = 444, female = 489), [2]n = 942 (male = 449, female = 493)

Table II: Percentile distributions for retinol (ng/mL), beta-carotene (ng/mL), α-tocopherol (µg/mL), and vitamin C (mg/L) of apparently healthy male European adolescents aged 12.5–17.49 y

		Retinol (ng/mL)	β-carotene (ng/mL)	α-tocopherol (µg/mL)	Vitamin C (mg/L)
	n	444	449	444	501
Percentile	2.5	196.70	54.74	6.00	2.53
	5	216.03	71.06	6.34	3.43
	10	243.10	90.01	7.03	5.38
	25	285.56	131.36	7.97	8.18
	50	353.72	192.47	9.39	10.36
	75	417.18	275.44	10.76	12.09
	95	550.71	578.49	13.06	14.85
	97.5	629.50	707.21	13.92	16.61

Table III: Percentile distributions for retinol (ng/mL), beta-carotene (ng/mL), α-tocopherol (µg/mL), and vitamin C (mg/L) of apparently healthy female European adolescents aged 12.5–17.49 y

		Retinol (ng/mL)	β-carotene (ng/mL)	α-tocopherol (µg/mL)	Vitamin C (mg/L)
	n	488	493	488	553
Percentile	2.5	194.07	71.17	6.49	3.35
	5	210.85	80.52	7.10	4.90
	10	236.90	100.29	7.76	6.19
	25	277.02	148.37	8.74	8.65
	50	326.94	215.56	9.98	10.87
	75	405.59	311.60	11.58	12.63
	95	566.76	600.25	14.30	15.97
	97.5	622.61	712.57	15.16	16.81

Table IV: Percentile distributions for retinol (ng/mL) of apparently healthy European adolescents presented by age groups

Retinol (ng/mL		male							
Age groups	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	112	145.2	188.1	203.8	251.4	310.4	366.7	461.0	496.6
14.0–14.99	110	197.4	222.8	242.8	277.4	331.0	392.6	520.4	610.9
15.0–15.99	118	218.9	235.5	262.3	307.4	371.0	416.3	550.4	611.4
16.0–17.49	104	223.1	281.9	285.5	335.0	423.4	491.0	679.8	692.6
		female							
	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	129	189.3	200.9	224.8	270.7	309.3	361.6	484.2	589.8
14.0–14.99	125	174.3	202.8	220.8	274.2	314.2	403.8	525.6	601.3
15.0–15.99	130	195.6	211.7	238.0	278.4	333.2	421.7	558.2	629.5
16.0–17.49	113	200.5	237.5	254.7	288.4	351.8	449.3	666.1	785.8

Table V: Percentile distributions for β-carotene (ng/mL) of apparently healthy European adolescents presented by age groups

β-carotene (ng/mL)		male							
Age groups	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	111	44.2	69.6	91.2	128.5	205.7	296.8	529.2	879.3
14.0–14.99	116	51.5	62.3	89.2	134.2	185.5	262.6	601.8	714.3
15.0–15.99	118	46.9	64.2	84.2	116.1	181.3	251.8	574.1	615.1
16.0–17.49	104	72.9	84.8	99.4	156.1	204.9	288.5	638.6	980.9
		female							
	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	121	57.3	72.0	90.1	132.2	197.9	299.7	579.7	728.9
14.0–14.99	126	73.7	80.5	98.4	161.7	235.0	314.3	615.1	704.8
15.0–15.99	132	67.3	72.3	101.7	142.3	191.1	294.2	512.5	534.7
16.0–17.49	114	74.9	88.7	105.9	159.5	234.2	346.6	721.9	957.2

Figure 1 shows smoothed centile curves (P5, P25, P50, P75, P95) for retinol, α-tocopherol, vitamin C, and β-carotene by sex and age.

Discussion

Scientific knowledge about antioxidant vitamin status in adolescents in both developed and developing countries is scarce. In the review by Lambert *et al.* [22] concerning the nutritional status of European adolescents, antioxidant vitamins were barely mentioned. In the more recent review performed by our research group, we confirmed the scarcity of available data [18]. Only a few studies about antioxidant vitamins status in adolescents have been carried out in the last decade in some European countries, but comparison of the data is not always possible due to the use of different methods, age-group categories, and reduced number of subjects [18]. As there are no representative data on the metabolic and biochemical status of healthy adolescents, reference values are missing for many blood parameters. Often, reference values for adults have been used to establish limits for vitamin levels in adolescents [2, 9].

In order to be able to define these reference values, data must be analyzed by both age and gender. It is more than reasonable to suppose that growth and development will have an influence on blood con-

Table VI: Percentile distributions for α-tocopherol (μg/mL) of healthy European adolescents presented by age groups

α-tocopherol (μg/mL)		male							
Age groups	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	112	5.8	6.1	6.6	8.0	9.3	10.6	13.2	14.2
14.0–14.99	110	6.6	6.9	7.3	8.2	9.4	10.5	12.8	14.1
15.0–15.99	118	5.3	6.1	7.1	7.9	9.6	11.1	13.4	14.5
16.0–17.49	104	6.2	6.6	7.1	7.9	9.4	10.4	12.6	13.3
		female							
	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	120	6.2	6.7	7.6	8.7	9.5	11.0	13.8	15.4
14.0–14.99	126	6.2	7.0	7.7	8.8	10.1	11.3	14.2	15.0
15.0–15.99	130	6.9	7.8	8.2	9.0	10.7	11.8	14.2	15.4
16.0–17.49	113	6.4	7.2	7.7	8.6	9.9	12.0	14.8	15.5

Table VII: Percentile distributions for vitamin C (mg/L) of apparently healthy European adolescents presented by age groups

Vitamin C (mg/L)		male							
Age groups	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	128.0	2.5	2.8	4.0	7.6	10.4	12.4	16.1	17.8
14.0–14.99	127.0	1.7	2.8	5.0	8.0	10.4	12.4	14.5	15.6
15.0–15.99	128.0	2.8	3.4	5.6	8.3	10.2	12.0	14.7	17.1
16.0–17.49	119.0	3.8	5.2	6.6	8.5	10.1	11.7	14.7	16.0
		female							
	n	P 2.5	P 5	P 10	P 25	P 50	P 75	P 95	P 97.5
12.5–13.99	143.0	3.2	4.5	5.9	8.0	10.6	13.0	16.0	16.8
14.0–14.99	142.0	3.2	4.9	6.6	9.2	11.0	13.1	16.2	18.8
15.0–15.99	141.0	3.6	4.6	5.9	8.5	11.0	12.4	16.0	17.1
16.0–17.49	127.0	3.7	5.2	6.3	8.8	10.9	12.4	15.5	17.1

centrations. In fact, several smaller studies have dealt with this issue.

In the same way as in our HELENA data and confirmed by the LMS percentile curves (Figure 1), retinol concentrations increased with age in both genders in all analyzed studies [11, 14, 19, 20, 33–35]. Plasma β carotene levels seemed to be more stable through the age span in several studies performed on French adolescents [19, 20, 34]. On the contrary, in British adolescents, β-carotene concentrations increased with age in both sexes ($p < 0.01$) [33]. In our study, an increase of β-carotene concentrations with age was observed only in girls ($p < 0.05$). Alpha-tocopherol concentrations remained stable with increasing age in French [19, 20, 34] and British [33] adolescents, whereas age was found to be a significant predictor of plasma α-tocopherol concentrations in Swiss subjects aged 0.4 to 38.7 years [11]. In this last study, the big age-range of the analyzed subjects could have influenced the results. In our study, only in females a trend to increasing α-tocopherol levels can be observed (Figure 1).

In relationship to gender, in the 509 French adolescents studied by Herbeth *et al.* [14], boys had significantly higher retinol concentrations than girls ($p < 0.001$), similar to what we observed for the oldest age group. Gender differences for β-carotene and α-tocopherol were measured in an Austrian study. Boys had significant higher concentrations than girls ($p < 0.001$) [21]. These findings are contrary to ours, as we observed significantly higher α-tocopherol concentrations in girls than in boys ($p < 0.001$), but no differences in β-carotene concentrations.

In reference to vitamin C, our results showed higher vitamin C concentrations in females compared with males. Within the National Health and Nutrition Examination Survey (NHANES, 2003–2004) vitamin C concentrations in youth were investigated and also presented in percentiles, which were similar to our results. They also observed significantly higher vitamin C levels in females compared to males in the age group 12–19 years (males vs. females: 50.7 to 54.8 μmol/L, $p<0.05$) [7]. Hercberg *et al.* [19] also found this relationship in vitamin C by sex, but on the contrary, Gregory *et al.* [36] did not find any significant difference between males and females, but did find a decrease of vitamin C with increasing age in boys. Focusing on our data, the mean for vitamin C is slightly higher than the mean found in other studies [19, 36].

The HELENA study has several strengths. A harmonized and standardized methodology, which was tested previously in a pilot study, guaranteed high-quality data. The sampling procedure and the strict standardization of the field work among the countries involved in the study avoided to a great extend the kind of confounding bias due to inconsistent protocols and different laboratory methods, which typically makes comparing results from isolated studies difficult. The main contribution of the present data is to give for the first time a global overview of adolescent antioxidant vitamin status in Europe. In the absence of reference values and specific cut-off points for this age group, percentile distribution as presented can be used in clinics and further research. It is important to remember that current plasma levels of vitamins in the adolescent population do not necessary mean that these levels are the most adequate ones from the biological point of view.

Conclusions

The present study provides data on the distribution of retinol, α-tocopherol, β-carotene, and vitamin C concentrations in European adolescents. Differences by gender were observed, with females showing higher vitamin C and α-tocopherol values compared with males, but with males showing higher retinol values compared to females. Retinol serum concentrations increased significantly according to age. The HELENA percentile distribution is in agreement with data coming from smaller studies and could be used as reference values to characterise vitamin status of European adolescents.

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*Helena Study Group

Co-ordinator: Luis A. Moreno

Core Group members: Luis A. Moreno, Fréderic Gottrand, Stefaan De Henauw, Marcela González-Gross, Chantal Gilbert.

Steering Committee: Anthony Kafatos (President), Luis A. Moreno, Christian Libersa, Stefaan De Henauw, Jackie Sáchez, Fréderic Gottrand, Mathilde Kesting, Michael Sjostrom, Dénes Molnár, Marcela González-Gross, Jean Dallongeville, Chantal Gilbert, Gunnar Hall, Lea Maes, Luca Scalfi.

Project Manager: Pilar Meléndez

1. Universidad de Zaragoza (Spain): Luis A. Moreno, Jesús Fleta, José A. Casajús, Gerardo Rodríguez, Concepción Tomás, María I. Mesana, Germán Vicente-Rodríguez, Adoración Villarroya, Carlos M. Gil, Ignacio Ara, Juan Revenga, Carmen Lachen, Juan Fernández Alvira, Gloria Bueno, Aurora Lázaro, Olga Bueno, Juan F. León, Jesús Mª Garagorri, Manuel Bueno, Juan Pablo Rey López, Iris Iglesia, Paula Velasco, Silvia Bel.

2. Consejo Superior de Investigaciones Científicas (Spain): Ascensión Marcos, Julia Wärnberg, Esther Nova, Sonia Gómez, Esperanza Ligia Díaz, Javier Romeo, Ana Veses, Mari Angeles Puertollano, Belén Zapatera, Tamara Pozo.

3. Université de Lille 2 (France): Laurent Beghin, Christian Libersa, Frédéric Gottrand, Catalina Iliescu, Juliana Von Berlepsch.

4. Research Institute of Child Nutrition Dortmund, Rheinische Friedrich-Wilhelms-Universität Bonn

(Germany): Mathilde Kersting, Wolfgang Sichert-Hellert, Ellen Koeppen.
5. Pécsi Tudományegyetem (University of Pécs) (Hungary): Dénes Molnar, Eva Erhardt, Katalin Csernus, Katalin Török, Szilvia Bokor, Mrs. Angster, Enikö Nagy, Orsolya Kovács, Judit Repásy.
6. University of Crete School of Medicine (Greece): Anthony Kafatos, Caroline Codrington, María Plada, Angeliki Papadaki, Katerina Sarri, Anna Viskadourou, Christos Hatzis, Michael Kiriakakis, George Tsibinos, Constantine Vardavas Manolis Sbokos, Eva Protoyeraki, Maria Fasoulaki.
7. Institut für Ernährungs- und Lebensmittelwissenschaften –Ernährungphysiologie. Rheinische Friedrich Wilhelms Universität (Germany): Peter Stehle, Klaus Pietrzik, Marcela González-Gross, Christina Breidenassel, Andre Spinneker, Jasmin Al-Tahan, Miriam Segoviano, Anke Berchtold, Christine Bierschbach, Erika Blatzheim, Adelheid Schuch, Petra Pickert.
8. University of Granada (Spain): Manuel J. Castillo, Ángel Gutiérrez, Francisco B. Ortega, Jonatan R Ruiz, Enrique G. Artero, Vanesa Espana-Romero, David Jiménez-Pavón, Palma Chillón.
9. Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Italy): Davide Arcella, Elena Azzini, Emma Barrison, Noemi Bevilacqua, Pasquale Buonocore, Giovina Catasta, Laura Censi, Donatella Ciarapica, Paola D'Acapito, Marika Ferrari, Myriam Galfo, Cinzia Le Donne, Catherine Leclercq, Giuseppe Maiani, Beatrice Mauro, Lorenza Mistura, Antonella Pasquali, Raffaela Piccinelli, Angela Polito, Raffaella Spada, Stefania Sette, Maria Zaccaria.
10. University of Napoli "Federico II" Dept of Food Science (Italy): Luca Scalfi, Paola Vitaglione, Concetta Montagnese.
11. Ghent University (Belgium): Ilse De Bourdeaudhuij, Stefaan De Henauw, Tineke De Vriendt, Lea Maes, Christophe Matthys, Carine Vereecken, Mieke de Maeyer, Charlene Ottevaere.
12. Medical University of Vienna (Austria): Kurt Widhalm, Katharina Phillipp, Sabine Dietrich, Birgit Kubelka Marion Boriss-Riedl.
13. Harokopio University (Greece): Yannis Manios, Eva Grammatikaki, Zoi Bouloubasi, Tina Louisa Cook, Sofia Eleutheriou, Orsalia Consta, George Moschonis, Ioanna Katsaroli, George Kraniou, Stalo Papoutsou, Despoina Keke, Ioanna Petraki, Elena Bellou, Sofia Tanagra, Kostalenia Kallianoti, Dionysia Argyropoulou, Katerina Kondaki, Stamatoula Tsikrika, Christos Karaiskos.
14. Institut Pasteur de Lille (France): Jean Dallongeville, Aline Meirhaeghe.
15. Karolinska Institutet (Sweden): Michael Sjöstrom, Patrick Bergman, María Hagströmer, Lena Hallström, Marten Hallberg, Eric Poortvliet, Julia Wärnberg, Nico Rizzo, Linda Beckman, Anita Hurtig Wennlöf, Emma Patterson, Lydia Kwak, Lars Cernerud, Per Tillgren, Stefaan Sörensen.
16. Asociación de Investigación de la Industria Agroalimentaria (Spain): Jackie Sánchez-Molero, Elena Picó, Maite Navarro, Blanca Viadel, José Enrique Carreres, Gema Merino, Rosa Sanjuán, María Lorente, María José Sánchez, Sara Castelló.
17. Campden & Chorleywood Food Research Association (United Kingdom): Chantal Gilbert, Sarah Thomas, Elaine Allchurch, Peter Burguess.
18. SIK – Institutet foer Livsmedel och Bioteknik (Sweden): Gunnar Hall, Annika Astrom, Anna Sverkén, Agneta Broberg.
19. Meurice Recherche & Development asbl (Belgium): Annick Masson, Claire Lehoux, Pascal Brabant, Philippe Pate, Laurence Fontaine.
20. Campden & Chorleywood Food Development Institute (Hungary): Andras Sebok, Tunde Kuti, Adrienn Hegyi.
21. Productos Aditivos SA (Spain): Cristina Maldonado, Ana Llorente.
22. Cárnicas Serrano SL (Spain): Emilio García.
23. Cederroth International AB (Sweden): Holger von Fircks, Marianne Lilja Hallberg, Maria Messerer.
24. Lantmännen Food R&D (Sweden): Mats Larsson, Helena Fredriksson, Viola Adamsson, Ingmar Börjesson.
25. European Food Information Council (Belgium): Laura Fernández, Laura Smillie, Josephine Wills.
26. Universidad Politécnica de Madrid (Spain): Marcela González-Gross, Jara Valtuena, Ulrike Albers, Raquel Pedrero, Agustín Meléndez, Pedro J. Benito, David Canada, David Jiménez-Pavón, Alejandro Urzanqui, Juan Carlos Ortiz, Francisco Fuentes, Juan José Gómez Lorente, Rosa María Torres, Paloma Navarro.

References

1. Biesalski, H.-K., Bischoff, S.C. and Puchstein, C. (2010) Ernährungsmedizin: Nach dem Curriculum Ernährungsmedizin der Bundesärztekammer und der DGE 4. vollst. überarb. u. erw. Aufl. ed. Stuttgart: Thieme.

2. Koletzko, B., de la Gueronniere, V., Toschke, A.M. and von Kries, R. (2004) Nutrition in children and adolescents in Europe: what is the scientific basis? Introduction. Br. J. Nutr. 92, Suppl 2, S67–73.

3. Molnar, D., Decsi, T.and Koletzko, B. (2004) Reduced antioxidant status in obese children with multimetabolic syndrome. Int. J. Obes. Relat. Metab. Disord. 28 (10), 1197–202.

4. Board FaN. (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nikkel, Silicon, Vanadium, and Zinc. The National Academies Press, Washington, D.C.

5. World Health Organization, Nations FaAOotU. (2004)Vitamin and Mineral Requirements in Human Nutrition. World Health Organization (WHO).

6. Board FaN. (2000) Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. The National Academy Press, Washington, D.C.

7. Schleicher, R.L., Carroll, M.D., Ford, E.S. and Lacher, D.A. (2009) Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). Am. J. Clin. Nutr. 90 (5), 1252–63.

8. DACH. (2000) Referenzwerte für die Nährstoffzufuhr: Umschau/Braus GmbH, Frankfurt.

9. Prentice, A., Branca, F., Decsi, T., Michaelsen, K.F., Fletcher, R.J., Guesry, P. et al. (2004) Energy and nutrient dietary reference values for children in Europe: methodological approaches and current nutritional recommendations. Br. J. Nutr. 92 Suppl 2, S83–146.

10. Rolland-Cachera, M.F., Bellisle, F. and Deheeger, M. (2000) Nutritional status and food intake in adolescents living in Western Europe. Eur. J. Clin. Nutr. 54 Suppl 1, S41–6.

11. Winklhofer-Roob, B.M., van't Hof, M.A. and Shmerling, D.H. (1997) Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences. Clin. Chem. 43 (1), 146–53.

12. Aeberli, I., Molinari, L., Spinas, G., Lehmann, R., l'Allemand, D. and Zimmermann, M.B. (2006) Dietary intakes of fat and antioxidant vitamins are predictors of subclinical inflammation in overweight Swiss children. Am. J. Clin. Nutr. 84 (4), 748–55.

13. Moreno, L. (2008) Adolescence. In:. Pediatric Nutrition in Practice. (Koletzko, B., ed.) p. 114–117, Karger, Basel.

14. Herbeth, B., Spyckerelle, Y. and Deschamps, J.P. (1991) Determinants of plasma retinol, beta-carotene, and alpha-tocopherol during adolescence. Am. J. Clin. Nutr. 54 (5), 884–9.

15. Ortega, F.B., Ruiz, J.R., Castillo, M.J., Moreno, L.A., Gonzalez-Gross, M., Warnberg, J. *et al.* (2005) Low Level of Physical Fitness in Spanish Adolescents. Relevance for Future Cardiovascular Health (AVENA Study). Rev. Esp. Cardiol. 58 (8), 898–909.

16. Moreno, L.A., Joyanes, M., Mesana, M.I., Gonzalez-Gross, M., Gil, C.M., Sarria, A. *et al.* (2003) Harmonization of anthropometric measurements for a multicenter nutrition survey in Spanish adolescents. Nutrition 19 (6), 481–6.

17. Ortega, R.M., Mena, M.C., Faci, M., Santana, J.F. and Serra-Majem, L. (2001) Vitamin status in different groups of the Spanish population: a meta-analysis of national studies performed between 1990 and 1999. Public Health Nutr. 4 (6 A), 1325–9.

18. Valtuena, J., Breidenassel, C., Folle, J. and González-Gross, M. (2010) Retinol, β-carotene, α tocopherol and vitamin D status in European adolescents. Regional differences and variability. A review. Nutr. Hosp., 26 (2): 280–8.

19. Hercberg, S., Preziosi, P., Galan, P., Devanlay, M., Keller, H., Bourgeois, C. *et al.* (1994) Vitamin status of a healthy French population: dietary intakes and biochemical markers. Int. J. Vitam. Nutr. Res. 64 (3), 220–32.

20. Malvy, J.M., Mourey, M.S., Carlier, C., Caces, P., Dostalova, L., Montagnon, B. *et al.* 1989) Retinol, beta-carotene and alpha-tocopherol status in a French population of healthy children. Int. J. Vitam. Nutr. Res. 59 (1), 29–34.

21. Marktl, W., Rudas, B. and Brubacher, G. (1982) The vitamin status of Viennese school children aged 11–12 years. Int. J. Vitam. Nutr. Res.52(2),198–206.

22. Lambert, J., Agostoni, C., Elmadfa, I., Hulshof, K., Krause, E., Livingstone, B. (2004) *et al.* Dietary intake and nutritional status of children and adolescents in Europe. Br. J. Nutr. 92 Suppl 2, S147–211.

23. De Henauw, S., Gottrand, F., De Bourdeaudhuij, I., Gonzalez-Gross, M., Leclercq, C., Kafatos, A. *et al.* (2007) Nutritional status and lifestyles of adolescents in a public health perspective. The HELENA Project – Healthy Lifestyle in Europe by Nutrition in Adolescence. J. Publ. Health 15, 10.

24. Moreno, L.A., De Henauw, S., Gonzalez-Gross, M., Kersting, M., Molnar, D., Gottrand, F. *et al.* (2008) Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int. J. Obes. (Lond) 32 Suppl 5, S4–11.

25. Moreno, L.A., Gonzalez-Gross, M., Kersting, M., Molnar, D., de Henauw, S., Beghin, L. *et al.* (2008) Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr.11 (3), 288–99.

26. European parliament directive 2001/20/CE of the European parliament and council of 4 April 2001. Official Journal 2001, L121, pp. 34–44.

27. Beghin, L., Castera, M., Manios, Y., Gilbert, C.C., Kersting, M., De Henauw, S. *et al.* (2008) Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. Int. J. Obes. (Lond) 32 Suppl 5, S12–8.

28. Gonzalez-Gross, M., Breidenassel, C., Gomez-Martinez, S., Ferrari, M., Beghin, L., Spinneker, A. *et al.* (2008) Sampling and processing of fresh blood samples within a European multicenter nutritional study: evaluation of biomarker stability during transport and storage. Int. J. Obes. (Lond) 32 Suppl 5, S66–75.

29. Nagy, E., Vicente-Rodriguez, G., Manios, Y., Beghin, L., Iliescu, C., Censi, L. *et al.* (2008) Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. Int. J. Obes. (Lond) 32 Suppl 5, S58–65.

30. Tanner, J.M. and Whitehouse, R.H. (1976) Clinical longitudinal standards for height, weight, height velocity and stages of puberty. Arch. Dis. Child. 51, 170–179.

31. Cole, T.J., Freeman, J.V. and Preece, M.A. (1998) British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat. Med. 17 (4), 407–29.

32. Cole, T.J. and Green, P.J. (1992) Smoothing reference centile curves: the LMS method and penalized likelihood. Stat. Med. 11 (10), 1305–19.

33. Gregory, J., Lowe, S., Bates, C., Prentice, A., Jackson, L., Smithers, G. *et al.* (2000) National Diet and Nutrition Survey: young people aged 4–18 years. Volume 1: Report of the diet and nutrition survey. London.

34. Drott, P., Meurling, S. and Gebre-Medhin, M. (1993) Interactions of vitamins A and E and retinol-binding protein in healthy Swedish children–evidence of thresholds of essentiality and toxicity. Scand. J. Clin. Lab Invest. 53 (3), 275–80.

35. Malvy, D.J., Burtschy, B., Dostalova, L., Amedee-Manesme, O. (1993) Serum retinol, beta-carotene, alpha-tocopherol and cholesterol in healthy French children. Int. J. Epidemiol. 22 (2), 237–46.

36. Gregory, J.L.S., Bates, C.J., Prentice, A., Jackson, L.V., Smithers, G., Wenlock, R. and Farron, M. (2000) National Diet and Nutrition Survey: young people aged 4–18 years. Volume 1: Report of the diet and nutrition survey. London.

Dr. Christina Breidenassel

Institut für Ernährungs- und
Lebensmittelwissenschaften – IEL
Rheinische Friedrich-Wilhelms Universität
Endenicher Allee 11–13
DE-53115 Bonn
Germany
c.breidenassel@uni-bonn.de