British Journal of Nutrition (2012), **107**, 755–764
doi:10.1017/S0007114511003527

Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study

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(Received 12 November 2010 – Revised 23 May 2011 – Accepted 31 May 2011 – First published online 17 August 2011)

Abstract

An adequate vitamin D status is essential during childhood and adolescence, for its important role in cell growth, skeletal structure and development. It also reduces the risk of conditions such as CVD, osteoporosis, diabetes mellitus, infections and autoimmune disease. As comparable data on the European level are lacking, assessment of vitamin D concentrations was included in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study. Fasting blood samples were obtained from a subsample of 1006 adolescents (470 males; 46·8 %) with an age range of 12·5–17·5 years, selected in the ten HELENA cities in the nine European countries participating in this cross-sectional study, and analysed for 25-hydroxycholecalciferol (25(OH)D) by ELISA using EDTA plasma. As specific reference values for adolescents are missing, percentile distribution were computed by age and sex. Median 25(OH)D levels for the whole population were 57·1 nmol/l (5th percentile 24·3 nmol/l, 95th percentile 99·05 nmol/l). Vitamin D status was classified into four groups according to international guidelines (sufficiency/optimal levels ≥75 nmol/l; insufficiency 50–75 nmol/l; deficiency 27·5–49·99 nmol/l and severe deficiency <27·5 nmol/l). About 80 % of the sample had suboptimal levels (39 % had insufficient, 27 % deficient and 15 % severely deficient levels). Vitamin D concentrations increased with age ($P<0·01$) and tended to decrease according to BMI. Geographical differences were also identified. Our study results indicate that vitamin D deficiency is a highly prevalent condition in European adolescents and should be a matter of concern for public health authorities.

Key words: Adolescents: Vitamin D: Prevention: Europe

Adolescents are considered as a risk group for malnutrition because of their increasing needs of nutrients and energy for adequate growth and development that vary with age[1–3]. Specifically different levels of vitamin D deficiency at these early ages could be considered a risk factor for osteomalacia[4–6], impaired cognitive function and concentration problems[7], hyperactivity[8] and immune system deficiency[4]. Inadequate vitamin D levels have also been related to other diseases such as diabetes, multiple sclerosis and cancer[9–11]. One of the most important applications

Abbreviations: 25(OH)D, 25-hydroxycholecalciferol; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence.

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of vitamin D assessment in adolescence is related to bone health[12] and reaching an optimal peak bone mass in adulthood[13,14]. The main sources of vitamin D are food intake and subcutaneous skin synthesis, under UV light (290–315 nm) exposure. However, due to the geographical situation of our continent, vitamin D synthesis may not compensate for a low nutritional intake[15]. Subclinical vitamin D deficiency could remain undetected as it is not routinely screened for in these population groups. The main circulating vitamin D metabolite, 25-hydroxycholecalciferol (25(OH)D), has been proposed as the best indicator of vitamin D status, because it represents not only the amount consumed through diet and supplements but also the subcutaneous synthesis[15–18].

Scientific knowledge about vitamin D status in the period of adolescence in both developed and developing countries is still scarce. Several studies on the status of vitamin D in European adolescents have been carried out in the last decade, but only a few have used a significant number of subjects. As we have recently reviewed[19], comparison of the data is not always possible due to the use of different age ranges, different methods, different ways of presenting them in the different studies and a lack of consensus on cut-off levels. Proposed deficient and sufficient 25(OH)D vitamin concentrations vary from 20 to 100 nmol/l depending on the studies[20–22]. While there are no universally accepted blood 25(OH)D thresholds to define adequacy in adolescents, the following set has been proposed: concentrations of 25(OH)D less than 75 nmol/l as insufficient, concentrations less than 50 nmol/l as deficient and severe vitamin D deficiency when values are less than 27·5 nmol/l[23]. The proposed reference value for insufficiency in children (<75 nmol/l) has been extrapolated from adult data[18].

One of the main aims of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study was to provide, for the first time, comparable data about micronutrient status in European adolescents[24]. The main objective of the present study was to describe vitamin D status in adolescents and to analyse vitamin plasma concentrations by sex, age and weight status, thus contributing to establishing reference values that are not available for the adolescent population[25,26].

Subjects and methods

Subjects, recruitment and study design

The HELENA cross-sectional study was a multi-centre cross-sectional study aiming to obtain reliable and comparable data from a random sample of 3000 European adolescents aged between 12·5 and 17·49 years on a broad battery of nutrition and health-related parameters[24,27]. Subjects were recruited by a school-based, multi-step, stratified random and cluster sampling selection. Criteria for city selection included geographic balance and the presence of an experienced research group. The sample size was calculated to establish distributions of relevant study variables. Exclusion criteria were limited to subjects who were not able to speak the local language, who were participating simultaneously in another clinical trial, who were aged <12·5 or >17·5 years and who had suffered from acute infection 1 week before the visit. Exclusion from the study was decided *a posteriori*, without the knowledge of the affected subjects, to avoid non-desirable situations, and so whole classes were included. A complete description of the design and implementation of the study has been published elsewhere[24].

In the same manner as described earlier, a subsample of 1006 adolescents was selected for blood sampling in the ten HELENA cities in nine European countries: Athens (Greece), Dortmund (Germany), Ghent (Belgium), Heraklion (Greece), Lille (France), Pecs (Hungary), Rome (Italy), Stockholm (Sweden), Vienna (Austria) and Zaragoza (Spain). The protocol was approved by the corresponding human research review committees of Bonn (Dortmund), Lille, Rome, Zaragoza, Athens, Heraklion, Pecs, Ghent, Stockholm and Vienna. The study was performed following the ethical guidelines of the Declaration of Helsinki 1964 (revision of Edinburgh 2000), Convention of Oviedo (1997), the Good Clinical Practice, and the legislation about clinical research in human subjects in each of the participating countries. Informed written consent was obtained from subjects and parents or guardians. A complete description of ethical issues and good clinical practice within the HELENA cross-sectional study has been published elsewhere[28].

Specimen collection and biochemical analyses

A specific handling, transport and traceability system for biological samples was developed for the HELENA study and has already been described by González-Gross *et al.*[29]. Blood samples were obtained between October 2006 and June 2007, and in October 2007 (see Annex). A blood-sampling calendar was developed to coordinate the fieldwork between the centres and the central laboratory at the University of Bonn (Institut für Ernährungs- und Lebensmittelwissenschaften (IEL); Bonn, Germany). The blood sampling date depended on local fieldwork planning, the agreement of the school, and availability and capacity of the lab at IEL. Fasting blood samples were collected by venepuncture at school between 08.00 and 10.00 hours. For the measurement of vitamin D, blood was collected in EDTA tubes and transported at room temperature to the central laboratory at IEL within 24 h. There it was centrifuged at 3500 rpm for 15 min at 4°C and the supernatant was stored at −80°C until assayed. The samples were kept stable for 24 h at room temperature (CV: 4·3 %).

Serum concentration of 25(OH)D is considered to be the most reliable measure of overall vitamin D status and thus can be used to determine whether a subject is vitamin D sufficient. Plasma 25(OH)D was analysed by ELISA using a kit (OCTEIA 25(OH)D) from Immunodiagnostic System (Frankfurt am Main, Germany) and measured with a Sunrise™ Photometer by TECAN (Männedorf, Germany). The IDS OCTEIA 25(OH)D kit is an enzyme immunoassay intended for the quantitative determination of 25(OH)D and other hydroxylated metabolites in human serum or plasma. Results are used in conjunction

with other clinical and laboratory data to assist the clinician in the assessment of vitamin D sufficiency. The sensitivity of this method is 5 nmol/l 25(OH)D and the variation is less than 6 %. The mean recovery of 25(OH)D is 101 %. The CV for the method was less than 1 %.

Physical examination

The adolescents had their height and weight measured by trained researchers in a standardised way: weight was recorded to the nearest 0·1 kg, using an electronic scale (type SECA 861; SECA, Hamburg, Germany) and height was recorded to the nearest 0·1 cm, using a telescopic height measuring instrument (type SECA 225). The BMI was calculated from their measured height and weight (BMI = weight divided by height squared, (kg/m^2)). International age- and sex-specific cut-off points[30,31] were used to assess BMI category (underweight/normal weight/overweight/obese). The complete description of the anthropometric measurements of the study has been published elsewhere[32]. A physician classified the adolescents into one of the five maturation stages described by Tanner & Whitehouse[33].

Statistical analysis

25(OH)D showed a normal histogram distribution. Descriptive statistics were performed and values are shown as mean, standard deviation, percentile, median, minimum and maximum. For this study, vitamin D status was classified into four groups (vitamin D sufficiency/optimal levels ≥ 75 nmol/l; insufficiency 50–75 nmol/l; deficiency 27·5–49·99 nmol/l and severe deficiency < 27·5 nmol/l) following international guidelines[18,34,35]. The differences between sex, age groups and BMI groups were analysed using one-way ANOVA. All the analyses were adjusted by a weighting factor to balance the sample according to the age and sex distribution of the theoretical sample, to guarantee representation of each of the stratified groups.

To provide percentile value curves for European adolescents, we analysed vitamin D data by maximum penalised likelihood using the least mean square statistical method for boys and girls separately[36,37]. We derived smoothed centile charts using the least mean square 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 (CV). For the construction of the percentile curves, data were imported into the LmsChartMaker software (version 2.3; by Tim Cole and Huiqi Pan, Harlow Healthcare, South Shields, Tyne and Wear, UK) and the L, M and S curves were estimated. The rest of the data were analysed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Descriptive characteristics and mean vitamin D concentrations of the study sample by age and sex are shown in Tables 1 and 2. Girls had slightly higher mean concentrations than boys. Prevalence rates of vitamin D status according to the aforementioned sufficient–deficient classification are shown in Fig. 1. Considering the cut-off set for adults at 75 nmol/l, approximately 80 % of the sample was below the optimal levels. A slightly higher percentage of females (22·2 %) had sufficient 25(OH)D concentrations compared to males (15·1 %). Regarding 25(OH)D deficiency (< 27·5 nmol/l), an equal and high proportion of males and females revealed this status (15 %).

There is a tendency of increasing 25(OH)D concentrations with increasing age for the whole group ($P < 0·001$), which is only significant in girls when the sample is split by sex ($P < 0·05$). Percentile distribution by age and sex for the whole sample is shown in Table 2. 25(OH)D sufficiency (> 75 nmol/l) is reached at lower percentiles with increasing age. That means that at increasing ages there are fewer subjects with insufficient 25(OH)D levels. Regarding deficiency, the fifth percentile of 25(OH)D in both males and females is close to the level of < 27·5 nmol/l for all ages.

Fig. 2 shows smoothed centile curves (P5, P25, P50, P75, P95) for 25(OH)D levels studied by age and sex. Concentrations were similar in boys and girls, although in boys, first a decrease and after the age of 14 years an increase is observed. In girls, the curves seem to indicate that the decrease comes before the age of 13 years, because at age 13 years a slightly progressive increase with age with a similar slope to that of the boys was observed. In both boys and girls, the trend to higher 25(OH)D levels is seen for those at P75 and P95, whereas at the other lower levels there is a trend to stability.

Table 1. Descriptive characteristics of participants

(Mean values, standard deviations and number of participants)

	All (*n* 1006)		Male (*n* 470)		Female (*n* 536)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	14·9	1·2	14·9	1·2	14·9	1·2
Sexual maturation: Tanner stages I/II/III/IV/V (%)	0/5/19/44/37		2/6/19/42/31		0/4/19/45/32	
Height (cm)	165·8	9·3	170·2	9·7	161·9	7·0
Weight (kg)	59·0	12·3	62·1	13·7	56·2	10·2
BMI (kg/m^2)	21·4	3·6	21·3	3·8	21·4	3·4
25(OH)D (nmol/l)	58·8	23·1	57·4	22·7	60·0	23·4

25(OH)D, 25-hydroxycholecalciferol.

Table 2. 25-Hydroxycholecalciferol concentrations by age and sex in European adolescents (nmol/l)
(Mean values, standard deviations, number of participants and percentiles)

	n	Mean	SD	P2·5	P5	P10	P25	P50	P75	P90	P95	P97·5
Total (*n* 1006)	1006	58·8	23·1	20·9	24·9	31·6	43·5	57·0	71·3	87·8	99·1	112·9
Males (*n* 470)†	470	57·4	22·7	21·6	24·3	32	42·6	56·0	69·1	86·7	96·4	107·4
Age 13 (years)	124	56·9	22·8	24·2	27·0	32·5	41·8	55·8	66·6	78·3	101·5	132·0
Age 14 (years)	124	55·6	20·6	22·5	24·9	31·7	42·3	53·5	65·9	81·9	91·2	96·4
Age 15 (years)	122	58·3	21·3	20·7	24·4	33·6	44·8	56·2	71·3	89·0	94·9	102·0
Age 16 (years)	99	59·3	26·5	20·4	22·6	24·9	43·0	59·6	70·8	92·3	107·6	121·9
Females (*n* 536)†	536	60·0	23·4	20·9	25·5	31·0	44·8	57·9	74·3	88·7	103·0	115·9
Age 13 (years)	133	52·1*	19·2	17·0	20·9	25·6	40·6	50·8	64·0	78·0	85·8	96·8
Age 14 (years)	140	62·5*	22·3	20·9	27·8	33·6	49·1	62·0	77·9	90·2	99·2	111·3
Age 15 (years)	143	59·6*	20·8	21·3	26·0	36·2	46·6	56·8	71·5	90·2	97·2	106·0
Age 16 (years)	120	66·2*	28·9	25·6	26·9	27·4	46·4	61·8	80·3	109·3	120·8	133·5

* Mean values were significantly different between the 13 years age group and the rest of the age groups ($P<0·05$).
† Four age groups: 13 years, age between 12·5 and 13·99 years; 14 years, age between 14 and 14·99 years; 15 years, age between 15 and 15·99 years; 16 years, age between 16 and 17·49 years.

When analysing the data according to BMI, a non-significant and progressive decrease of 25(OH)D concentrations with increasing BMI is observed, the lowest levels being observed in obese adolescents (equivalent to BMI $>30\,kg/m^2$; Table 3). The highest mean levels were for boys in the underweight group and for girls in the optimal weight group (66·6 (SD 28·9) and 61·1 (SD 23·5) nmol/l, respectively). Most of the adolescents had optimal weight status (BMI $20–25\,kg/m^2$).

Table 4 shows 25(OH)D levels by study centre, for the whole group split by sex. The highest levels were obtained in Rome, Athens, Vienna and Zaragoza, and the lowest levels were found in Dortmund, Heraklion and Ghent for the whole group, where the sampling procedure went on for most of the academic year. In none of the cities were mean levels above the proposed cut-off of 75 nmol/l. Girls had higher mean levels in all cities except for Athens, Pecs and Lille. Deficient levels (<50 nmol/l) were highest in Dortmund (62·9 % of the population) and Ghent (53·3 %), and lowest in Athens (25·7 %) and Rome (26·4 %) (data not shown).

Discussion

Since the publication of the results of the SENECA (Survey in Europe on Nutrition and the Elderly; a Concerted Action) study[38], where unexpectedly only 3·5 % of the analysed European elderly presented optimum 25(OH)D levels (>60 nmol/l), public health authorities have been concerned about the widespread 25(OH)D deficiency in the European population. To the best of our knowledge, the data obtained in the framework of the HELENA study are the first to aim at establishing descriptive 25(OH)D status in adolescents at a European level. According to the Institute of Medicine report 2011, vitamin D intake for bone health should correspond to

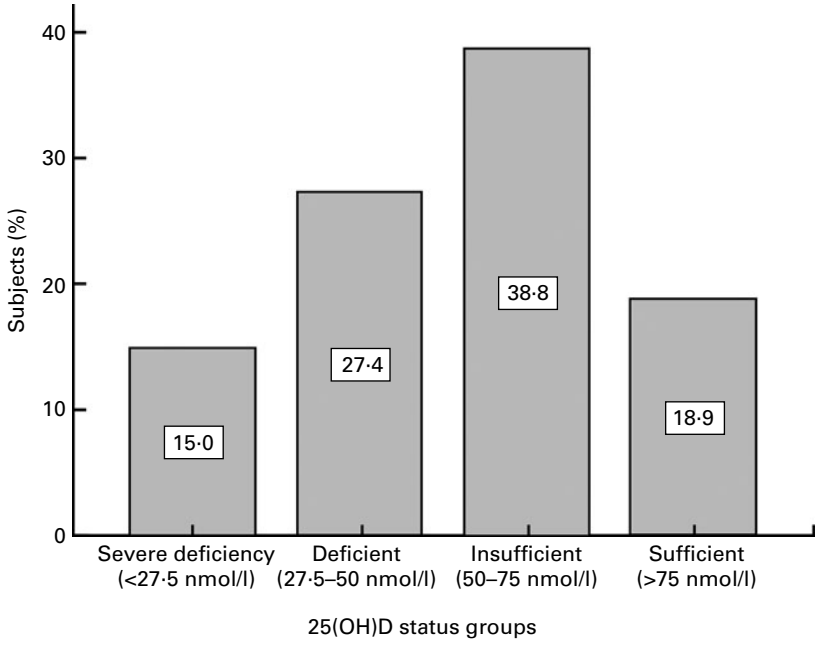

Fig. 1. 25-Hydroxycholecalciferol (25(OH)D) status classification.

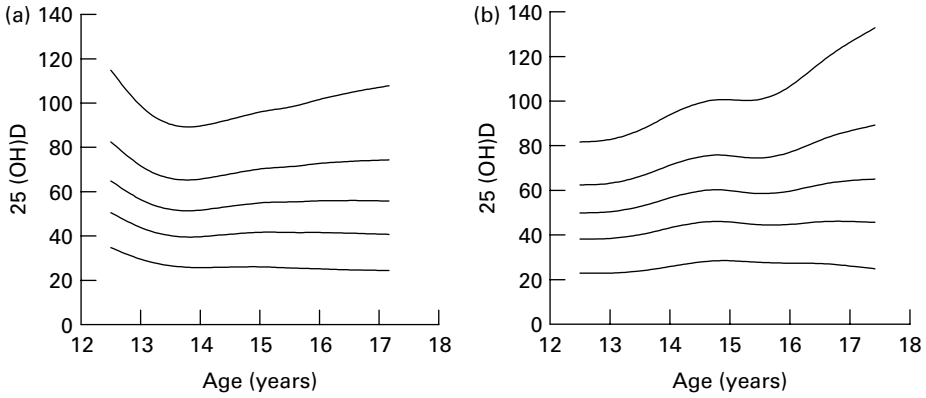

Fig. 2. Smoothed (least mean square method) centile curves (from the bottom to the top: P5, P25, P50, P75, P95) of 25-hydroxycholecalciferol (25(OH)D) plasma concentrations (nmol/l) in (a) males and (b) females.

a serum 25(OH)D level of at least 20 ng/ml (50 nmol/l)[23]. Our study results showed that approximately 40 % of the subjects had deficient levels lower than 50 nmol/l, 15 % with levels less than 27·5 nmol/l. None of the subjects had levels less than 10 nmol/l, which, according to the literature, elevates the risk for osteomalacia and rickets[14,39–42]. The HELENA percentile distribution is in agreement with data coming from other studies[20,24,27,28,43,44]. When analysing the percentile distribution of 25(OH)D we observed that the fifth percentile of 25(OH)D in both males and females, stratified by age, is close to the level of $<27{\cdot}5$ nmol/l for all ages. A general hypovitaminosis problem in adolescence varying from 13 to 72 % has already been postulated in studies performed in several European countries[20,24,27,28,43,44], the USA and Canada[45–49]. In a recent study published by Dong *et al.*[47], the overall prevalence of vitamin D insufficiency and deficiency in US children and adolescents was 56·4 and 28·8 %, respectively. All together, the high levels of vitamin D deficiency found in the present and other studies should be treated with caution. Regarding our percentile distribution, the median value of a 25(OH)D concentration in our European adolescents is close to 60 nmol/l, much lower than the optimal levels proposed of 75 nmol/l. Following Lanham-New *et al.*[50], any discussion of an 'optimal' serum 25(OH)D concentration needs to define 'optimal' with care since it is important to consider the normal distribution of requirements and the vitamin D needs for a wide range of outcomes. In addition, in the Rank Forum on Vitamin D, 2009, there was also some uncertainty about the strength of evidence for the need to aim for substantially higher concentrations (25(OH)D) concentrations >75 nmol/l[50].

Analysing vitamin D status by age, we have observed a steady increase in 25(OH)D concentrations with increasing age but which is only significant in girls. This is not in line with other published data. Koenig & Elmadfa[51] found a decrease in 25(OH)D serum concentrations in Austrian adolescents up to 14 years of age and a slow increase between the ages of 15 and 19 years. Similar findings were observed by Gregory *et al.*[22], with a significant reduction in 25(OH)D serum concentrations according to increasing age in adolescence. Dong *et al.*[47] concluded in their study that plasma 25(OH)D levels were not associated with age ($P=0{\cdot}460$). Conversely, Bonofiglio *et al.*[25] found higher 25(OH)D serum concentrations in post-menarcheal girls when compared with pre-menarcheal girls. These higher concentrations were explained as an increase in the binding protein of vitamin D because of higher oestrogen levels caused by menarche. This could also be the explanation for the differences

Table 3. 25-Hydroxycholecalciferol concentrations by BMI (kg/m^2) in European adolescents (nmol/l) (Mean values, standard deviations, number of participants, minimum and maximum values)

	BMI (kg/m^2)*	*n*	Mean†	SD	Minimum	Maximum
All (*n* 1006)	Low	58	61·1	26·9	13·7	140·0
	Optimal	728	59·3	23·7	12·1	174·0
	Overweight	167	56·9	20·3	11·4	120·8
	Obese	52	55·3	18·4	23·8	111·3
Males (*n* 470)	Low	26	66·6	28·9	20·7	138·1
	Optimal	331	57·1	23·7	12·2	174·0
	Overweight	82	57·0	17·1	21·3	97·9
	Obese	31	54·8	16·4	26·4	101·5
Females (*n* 536)	Low	32	56·8	24·9	13·8	140·0
	Optimal	397	61·1	23·5	12·1	160·8
	Overweight	86	56·8	23·0	11·4	120·8
	Obese	21	56·1	21·4	23·8	111·3

* BMI category calculated using polynomial from Cole *et al.*[36,37]. Four BMI groups: low ($<18{\cdot}5$ in adults), optimal (18·5–25 in adults), overweight (25–30 in adults), obese (>30 in adults).
† Mean values were not significantly different.

Table 4. Mean 25-hydroxycholecalciferol (25(OH)D) concentrations (nmol/l) in the Healthy Lifestyle in Europe by Nutrition in Adolescence cities (Mean values, standard deviations, minimum and maximum values)

	25(OH)D (nmol/l)									
	All		Male				Female			
	Mean	SD	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Rome in Italy	70·0	19·3	67·7	17·5	37·2	116·3	71·8	20·5	25·6	111·3
Athens in Greece	68·2	20·8	70·4	21·2	28·1	137·8	66·5	20·5	21·2	123·9
Vienna in Austria	63·7	31·7	58·2	31·0	15·1	173·9	67·2	31·9	17·6	137·3
Zaragoza in Spain	62·9	19·2	62·9	22·2	24·4	149·8	62·9	16·4	34·3	93·9
Stockholm in Sweden	58·7	23·1	52·4	20·1	21·2	131·9	64·6	24·3	12·1	140·0
Pecs in Hungary	57·8	20·0	60·9	22·6	24·2	138·0	55·6	17·8	25·5	119·8
Lille in France	54·9	24·5	60·2	27·1	12·4	139·6	51·1	21·9	11·4	90·2
Gent in Belgium	52·3	22·8	49·9	22·6	12·2	103·2	54·7	22·9	15·2	105·9
Heraklion in Crete	51·3	13·4	51·9	12·4	25·1	88·4	50·7	14·4	14·4	84·3
Dortmund in Germany	49·3	21·8	48·1	17·7	21·6	98·1	51·0	27·1	13·2	160·7

observed in the centile curves in Fig. 2, as Tanner stages differ and girls are on average 2 years in advance within the maturation process. In the US study by Yetley[45] deficiency percentage also increased with increasing age (1% for infants and children aged <11 years, 5% for adolescents aged 12–19 years and 6% for adults aged <20 years).

Several reports have observed a relationship between BMI and vitamin D concentrations[52]. An inverse, but not significant, relationship between 25(OH)D and BMI was found in the HELENA sample (Table 3). In the literature there are discrepancies regarding this issue. While several studies reported a significant and inverse relationship[46,53,54], others did not find any associations of BMI and/or fat mass with 25(OH)D levels in the paediatric population[55,56]. This may be attributed, in part, to BMI-based categorisation of obesity and the variations associated with growth and development. There are also studies reporting that obesity is associated with decreased bioavailability of dietary and cutaneously synthesised vitamin D. This may be secondary to the sequestration of vitamin D into a larger pool of adipose tissue[57].

As we have reviewed recently[19], both geographical and seasonal differences can be appreciated throughout Europe when analysing independent studies. Owing to the complex methodology and the multiple objectives of the HELENA study, no specific calendar for vitamin D sampling could be established, which would have contributed to getting a more in-depth appreciation of these aspects. Although not assessed in the study, dietary vitamin D intake and personal UV exposure habits may partly explain geographical differences in vitamin D status. Nevertheless, our study data are similar to those published by others[20,24,27,28,43,44], as the highest concentrations were observed in Rome, Athens and Zaragoza, and the lowest concentrations in Dortmund, Gent and Lille. The low mean concentrations observed in Heraklion could be due to seasonal influences. Because of local logistics, blood sampling in Heraklion was performed only in February and March, two winter months, while in the other centres, with the exception of Athens, blood sampling was distributed throughout the school year (see Annex). The low concentrations obtained in Heraklion could indicate a risk during the winter months even in the Mediterranean countries. This is in accordance with some researchers who have already emphasised the need to supplement vitamin D due to low 25(OH)D concentrations[58,59], especially during winter months. The high mean concentrations observed in Vienna need further analysis, which is out of the scope of this article. All in all, the detected geographical differences make it difficult to give common recommendations to improve vitamin D status in adolescents at the European level.

Increasing mean 25(OH)D blood levels up to 40 ng/ml would have a positive impact on reducing the direct and indirect economic burden of disease[60].

Apart from the aforementioned limitation, the HELENA study has several strengths. The sampling procedure and the strict standardisation of the fieldwork among the countries involved in the study avoided to a great extent the kind of confounding bias due to inconsistent protocols and different laboratory methods, which makes comparing results from isolated studies difficult. The main contribution of the present data is, for the first time, to give a global overview of adolescent vitamin D 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 blood concentrations of vitamins in the adolescent population do not necessary mean that these concentrations are the most adequate ones from the biological point of view. For a future study, serum parathyroid hormone concentrations should be included as in children and adolescents, the relationship between serum 25(OH)D and parathyroid hormone is less clear[61]. Owing to the complex and enormous amount of variables analysed in the HELENA project, parathyroid hormone could not be assessed. Considering the cut-offs used, deficiencies have been observed. Apart from an insufficiency, this could indicate that vitamin D concentrations in adolescents may be different from those of adults, making it necessary to establish general cut-offs for this micronutrient concentration in blood for the adolescence period.

In conclusion, our data give descriptive information about vitamin D status in European adolescents. Age, sex and weight status seem to have an influence on blood concentrations and should be taken into account. Our study results,

with the limitations described earlier, indicate that vitamin D deficiency is a highly prevalent condition in European adolescents and needs to be addressed by public health authorities.

Disclosure

The content of this paper reflects only the authors' view and the rest of HELENA study members are not responsible for it. The writing group takes sole responsibility for the content of this article.

Acknowledgements

The HELENA study has taken place with the financial support of the European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034). The content of this article reflects only the authors' views and the European Community is not liable for any use that may be made of the information contained therein. Additional support was received from the Spanish Ministry of Education (AGL2007-29784-E/ALI; AP-2005-3827) and Universidad Politécnica de Madrid (CH/018/2008). Many thanks to Adelheid Schuch for her contribution to laboratory work, to Laura Barrios for statistical assistance and to Diane Schofield for final English review of the manuscript. None of the authors had any conflict of interests. Contributions of each author: M. G.-G., L. A. M., M. K., S. D. H., F. G., A. K., Y. M., M. F. and P. S. designed the HELENA study and conducted research (recruitment of adolescents, collection of data, and blood sampling; J. V., C. B., E. A. conducted research and performed blood analysis; M. G.-G. and J. V. performed statistical analysis; M. G.-G., J. V., C. B., L. A. M., M. K. and P. S. wrote the paper. M. G.-G. and P. S. had primary responsibility for final content. All authors read and approved the final manuscript.

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British Journal of Nutrition

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British Journal of Nutrition

Annex. Blood sampling calendar: number of subjects (age range) per city, during the sampling period

	Month									
City	October 2006	November 2006	December 2006	January 2007	February 2007	March 2007	April 2007	May 2007	June 2007	October 2007
Athens		88 (12·6–16·9 years)	37 (13·1–15·4 years)							
Crete					23 (13·1–14·5 years)	69 (12·6–15·9 years)				
Dortmund				57 (12·5–17·3 years)			30 (12·8–16·8 years)		32 (14·2–16·7 years)	
Gent	29 (14·8–16·1 years)	16 (13·9–14·9 years)			20 (13·3–15·9 years)	13 (16·4–17·4 years)	15 (15·3–16·2 years)	22 (14·4–16·5 years)		
Lille			25 (12·5–13·4 years)			19 (13·2–14·6 years)		15 (14·3–15·1 years)	35 (12·9–15·9 years)	
Pecs		3 (14·8–16·8 years)				56 (12·6–17·3 years)				
Rome			17 (12·6–16·6 years)			14 (16·1–17·3 years)		45 (14·3–17·1 years)	9 (14·3–17·4 years)	19 (13·7–17·1 years)
Stockholm			18 (16·4–16·9 years)	40 (14·0–16·7 years)			38 (13·2–14·1 years)	33 (12·6–13·3 years)		
Vienna		41 (13·7–17·3 years)			20 (14·2–16·9 years)	16 (15·8–17·0 years)		43 (14·7–17·0 years)		
Zaragoza			19 (12·5–14·6 years)		15 (14·9–17·1 years)	38 (13·9–15·8 years)		37 (13·4–15·2 years)	15 (12·6–16·4 years)	