Cardiorespiratory fitness in males, and upper limbs muscular strength in females, are positively related with 25-hydroxyvitamin D plasma concentrations in European adolescents: the HELENA study

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Summary

**Background:** High prevalence of vitamin D insufficiency (<75 nmol/l) has been previously reported in European adolescents. Vitamin D deficiency has been related to physical fitness and adiposity but it is not clearly known whether this relationship applies to growing children and adolescents.

**Aim:** To determine how body composition and physical fitness are related to 25-hydroxyvitamin D [25(OH)D] concentrations in European adolescents.

**Design:** The HEalthy Lifestyle in Europe by Nutrition in Adolescence-CSS study was a multi-centre cross-sectional study.

**Methods:** Plasma 25(OH)D, body composition and physical fitness measures were obtained in 1006 European adolescents (470 males) aged 12.5–17.5 years. Stepwise regression and ANCOVA were performed by gender using 25(OH)D as dependent variable, with body composition, physical fitness as independent variables controlling for age, seasonality and latitude.

**Results:** For males, maximum oxygen consumption (VO_{2max}) \( (B = 0.189) \) and body mass index (BMI) \( (B = -0.124) \) were independently associated with 25(OH)D concentrations (both \( P < 0.05 \)). For females, handgrip strength \( (B = 0.168; \; P < 0.01) \) was independently associated with 25(OH)D concentrations. Those adolescents at lower BMI and high fitness score presented significant higher 25(OH)D concentrations than those at lower fitness score in the other BMI groups \( (P < 0.05) \).

**Conclusions:** Cardiorespiratory fitness and upper limbs muscular strength are positively associated with 25(OH)D concentrations in male and female adolescents, respectively. Adiposity in males and low fat free mass in females are related to hypovitaminosis D. The interaction between fitness and BMI has a positive effect on 25(OH)D concentrations. Therapeutic interventions to correct the high rates of vitamin D deficiency in adolescents should consider physical fitness.

Introduction

Vitamin D is essential for a vast number of physiologic processes, and thus adequate concentrations are necessary for advantageous optimal health. The knowledge of the role of vitamin D status in general health has increased in the last few years with the discovery of vitamin D receptors in multiple tissue types. Muscle and adipose tissue are among the first non-traditional vitamin D target organs identified.\(^1,2\) Vitamin D is believed to stimulate muscle cell proliferation and growth.\(^3\) Apart from bone metabolism, vitamin D also plays an important role in strengthening the immune system, improving cardiovascular health,\(^4\) protecting against certain cancers and possibly even enhancing athletic performance.\(^1,3-7\) In the last 10 years, its deficiency has been known to cause muscle weakness, hypotonia and prolonged time to peak muscle contraction, as well as prolonged time to muscle relaxation.\(^8\)

Obesity, expressed as excess of body fat, also has an adverse effect on vitamin D status but this has been analysed mostly in adults.\(^9-11\) Moreover, obese individuals only produce half the amount of vitamin D synthesized by non-obese individuals in response to sun exposure.\(^12\)

Despite these evidences it is not clear whether this relationship applies to growing children and adolescents, and how low 25-hydroxyvitamin D [25(OH)D] concentration is associated with body composition and physical fitness. In adolescents, complications from vitamin D insufficiency are often late-onset rather than immediate, given that the majority of teenagers with vitamin D insufficiency are completely asymptomatic, and this fact complicates diagnosis.\(^7\)

Previously we have reported a high prevalence of vitamin D deficiency in adolescents in Europe.\(^13,14\) 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. In the frame of a multi-analysis, vitamin D was identified as a potential conditional factor of physical fitness,\(^15\) but body composition has not been considered. A deeper understanding of this relationship is needed in this population group.

Therefore, the purpose of this study is to determine how body composition, muscular strength and cardiorespiratory fitness interact with vitamin D status in healthy European adolescents. All the contributions to identify any factors that may be associated with low vitamin D concentrations may help public health authorities to optimize vitamin D status across Europe.

Subjects and methods

**Subjects, recruitment and study design**

The HELENA-CSS 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 17.5 years on a broad battery of nutrition and health-related parameters. Subjects were recruited by a multi-stage random cluster sampling procedure, using schools as primary sampling units and classes as secondary sampling units. Criteria for city selection included geographic balance and presence of an experienced research group. The sample size was calculated according to stratified random sampling with proportional affixation to the size of the strata (SEX and AGE) and minimum variance (Neyman). A confidence level of 95% and the ±0.3 error in the worst of situations for the parameter body mass index (BMI) in the more general case of the study was chosen. On the city level, diversity of the sample with respect to cultural and socio-economic aspects was achieved by performing a random proportional distribution of all schools taking into account the site of the school (district/zone of the city) and the type of school (public or private). Exclusion criteria were limited to subjects who were not able to speak the local language, subjects participating simultaneously in another clinical trial, subjects aged <12.5 or >17.5 years and subjects having suffered from acute infection 1 week before the visit. Exclusions from the study were done a posteriori, without the knowledge of the affected subjects, to avoid non-desired situations. Therefore, whole classes were studied. The complete description of the design and implementation of the study has been described elsewhere.

Within the sampling procedure, a random subgroup of ~1000 adolescents (target number, 100 in each city) was foreseen to participate in the blood sampling. Finally, 1089 adolescents conform the valid HELENA blood subsample. For the present work, a subset of 1006 adolescents (470 males and 536 females, mean age of 14.7 ± 1.2 years) had valid data on 25(OH)D concentrations. This subgroup was recruited between October 2006 and October 2007 in 10 cities of nine different 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 Human Research Review Committee of the Universities of Bonn (Dortmund), Lille, Rome, Zaragoza, Athens, Heraklion, Pecs, Ghent and Vienna. The study has been 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 humans in each of the participating countries. Informed written consent was obtained from subjects and parents or guardians. Complete description of ethical issues and good clinical practice within the HELENA-CSS is described elsewhere.

**Specimen collection and biochemical analyses**

Fasting blood samples were collected by venipuncture at school between 8 and 10 o’clock in the morning from November 2006 to October 2007, during the whole academic year, excluding the summer period. For the measurement of vitamin D, blood was collected in EDTA tubes transported at room temperature to the central laboratory at University of Bonn (IEL) within 24 h. There it was centrifuged at 3500 rpm for 15 min at 4°C and the supernatant stored at −80°C until assayed. The complete methodology has been described elsewhere. Plasma or serum concentrations of 25(OH)D are considered to be the most reliable measure of overall vitamin D status and thus can be used to assess the vitamin D status. Plasma 25(OH)D was analysed by ELISA using a kit (OCTEIA 25-Hydroxy Vitamin D) from Immunodiagnostic System (Germany) and measured with a Sunrise™ Photometer by TECAN (Germany). The IDS OCTEIA 25-Hydroxy Vitamin 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 to be 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 below 6%. The mean recovery of 25(OH)D is 101%. The CV for the method was below 1%. The optimal concentrations for 25(OH)D were established as 375 nmol/l, following international guidelines.

**Seasonality**

The variable ‘blood drawing date’ was used to compute seasonality defined as following and similar to previous studies: winter (1; January through March), spring (2; April through June) and autumn (3; October through December). Blood drawing was performed only during the academic year. The complete methodology of the sampling calendar procedure has been described elsewhere.

**Latitude**

The latitude was also taken into account as a con-founder in the analyses. The latitude of each city was obtained from http://maps.google.es/. Latitudes of the involved cities were: Stockholm (59°33’ N), Athens (37°98’ N), Heraklion (35°33’ N), Rome (41°89’ N), Zaragoza (41°66’ N), Pecs (46°07’ N), Ghent (51°06’ N), Lille (50°63’ N), Dortmund
(51° 51’ N) and Vienna (48° 21’ N). To make use of this data, latitudes were added to the database as numeric variables with two decimals (i.e. Stockholm = 59.55) as previously described.15

**Anthropometric measurements**

The complete description of anthropometric measures was described elsewhere.23 Adolescents had their height and weight measured by trained researchers in a standardized way in light clothing and without shoes. The weight was recorded to the nearest 0.1 kg, using an electronic scale (Type SECA 861, UK). The height of the adolescents was recorded to the nearest 0.1 cm, using a telescopic height measuring instrument (Type SECA 225, UK). The BMI of the adolescents was calculated from their measured height and weight [BMI = weight divided by height squared (kg/m²)]. International age- and gender-specific cut-off points24,25 were used to assess BMI categories (underweight/normal weight/overweight/obese). Fat mass (FM) and fat free mass (FFM) were assessed my means of a classical tetrapolar technique (BIA 101 AKERN SRL, Pontassieve, FI, Italy). An index for these parameters was created (FMI and FFMI) dividing the mass of each one by the square of the height. Subjects were classified in tertiles according to their body composition, males and females separately.

**Physical fitness**

The health-related physical fitness components (i.e. cardiorespiratory fitness (CRF) and muscular fitness) were assessed by the physical fitness tests from the Eurofit battery, described elsewhere.26 The scientific were assessed by the physical fitness tests from the cardiorespiratory fitness (CRF) and muscular fitness) on 25(OH)D concentrations, body composition and fitness tests.

Stepwise multivariate linear regression was selected as a way of choosing predictors of a particular dependent variable on the basis of statistical criteria. It was conducted to examine the independent associations of body composition (BMI z-score, FMI and FFMI) and physical fitness (muscular strength and cardiorespiratory fitness) on 25(OH)D concentrations by sex, taking 25(OH)D concentrations as dependent variable. Each model always retained age, seasonality and latitude as confounders (Model 0), and all body composition and fitness tests were entered together into a stepwise regression model. The contribution of each variable to the variance of 25(OH)D concentrations is explained by adjusted $R^2$ in Table 3. It gives the most useful measure of the success of our model ($R^2 \times 100$ as a percentage of the variance). Variables first entered in the model are the ones more related with 25(OH)D concentrations. Following variables give an additional value in order of appearance, explaining the variance of 25(OH)D concentrations. The beta value ($β$) is the measure of how strongly each predictor variable influences 25(OH)D concentrations. Thus, the higher the beta value the greater the impact of the predictor variable on the criterion variable. Correlation between predictive values and 25(OH)D concentrations was also given by $R$ in Table 3.

**Statistical analysis**

Descriptive values are shown as mean ± standard deviation, unless otherwise stated. Independent samples $t$-test was used to analyse differences of 25(OH)D between sexes. Pearson’s correlation coefficients were computed between 25(OH)D concentrations, body composition and fitness tests.

ANCOVA was performed to analyse the differences in 25(OH)D concentrations according to the tertiles of body composition and physical fitness (mean and standard error). Age, seasonality and latitude were included as covariates.

All analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 15.0 for WINDOWS; SPSS, Chicago, IL, USA), and values of $P<0.05$ were considered statistically significant. Figures were performed using SigmaPlot (version 10.0 for WINDOWS; Systat Software, San José, CA, USA).

**Results**

Table 1 includes descriptive characteristics of the sample split by sex. Males presented higher FFMI and physical fitness performance than girls ($P<0.001$). On the contrary, females had higher FMI compared with males ($P<0.001$). The mean
values of 25(OH)D concentrations were under the optimal concentrations (<75 nmol/l) for both males and females (57.44 ± 22.67 and 59.99 ± 23.40 nmol/l, respectively).

Pearson’s correlation coefficients for the variables involved in this study are shown on Table 2. In males, VO2max \( (r = 0.108) \) was positively correlated with 25(OH)D \( (P < 0.05) \). In females, handgrip strength \( (r = 0.116) \), age \( (r = 0.181) \) and height \( (r = 0.120) \) were positively correlated with 25(OH)D \( (all \ P < 0.01) \).

Table 3 shows the results from the stepwise multivariate linear regression analysis stratified by gender. For males, linear regression of 25(OH)D showed that VO2max and BMI \( (B = 0.189; P < 0.01, B = -0.125; P < 0.05, \) respectively) were independently associated with 25(OH)D concentrations after controlling for seasonality, latitude and age, explaining the 24.3% of the variance. For females, only handgrip strength \( (B = 0.168; P < 0.01) \) was independently associated with 25(OH)D concentrations after controlling for the same factors and explaining the 20.3% of the variance.

Figure 1 shows 25(OH)D concentrations according to body composition variables adjusted for age, seasonality and latitude. In males, the lower the FFMI the higher the 25(OH)D concentrations \( (P < 0.01) \). In females, no significantly differences were found.

Figure 2 shows 25(OH)D concentrations according to muscular strength and CRF adjusted for age, seasonality and latitude. In males, the higher the performance in standing long jump the higher the 25(OH)D concentration \( (P < 0.05) \).

Figure 3 shows 25(OH)D concentrations according to BMI and fitness score by sex, adjusted for age, seasonality and latitude. In both males and females, those subjects at lower BMI and high fitness score presented significantly higher 25(OH)D concentrations than those at lower fitness score and than the other BMI groups \( (P < 0.05) \).

**Discussion**

There is more and more evidence that vitamin D status is associated to other health-related factors beyond skeletal metabolism.6,7 High prevalence...
rates (80%) of insufficiency have been identified in Europe among adolescents within the HELENA study.\textsuperscript{13} It is now important to understand factors influencing these low vitamin D concentrations found, to contribute to public health authorities to optimize vitamin D status across Europe. Therefore, there is a need to get deeper into the relationship of vitamin D with two important health markers as physical fitness and body composition in growing children and adolescents.

Our main results reveal that VO\textsubscript{2max} (positively) and BMI (negatively) are independently associated with 25(OH)D concentrations in males, after controlling for a relevant set of confounders, whereas handgrip strength as a measure of muscular strength, is positively associated with 25(OH)D concentrations in females. Regarding the ANCOVA results, the lower the FFMI and the performance in standing long jump, the higher the 25(OH)D concentrations in males. In both males and females, the interaction between fitness and BMI has a significant positive effect on 25(OH)D concentrations.

Low serum 25(OH)D levels could be associated with a number of adverse outcomes in the human musculoskeletal, innate immune and cardiovascular systems.\textsuperscript{30}

Moreover, both cardiovascular and muscular fitness have been previously reported as strong predictors of health.\textsuperscript{31} Strong evidence was found indicating that higher levels of cardiorespiratory fitness in childhood and adolescence are associated with a healthier cardiovascular profile later in life. Muscular strength improvements from childhood to adolescence are negatively associated with changes in overall adiposity. In addition, a healthier body composition in childhood and adolescence is associated with a healthier cardiovascular profile later in life and with a lower mortality risk. Those parameters should be taken together into account when analysing nutritional deficiencies in adolescence.

There are several studies in the literature assessing vitamin D status in children and adolescents but mainly focused on physical activity rather than physical fitness. In addition, not only BMI but also FM and FFM have been included in our study, which according to the literature are more appropriate for the growing period.\textsuperscript{7,32} Our results show that BMI and percentage of body fat in males are negatively associated with 25(OH)D concentrations. This reduction in 25(OH)D concentrations with increased adiposity is assumed to be due to enhanced sequestration of vitamin D in fat tissue.\textsuperscript{33} Vitamin D affects lipolysis and adipogenesis in human adipocytes through its role in regulating intracellular calcium concentrations and could then influence adiposity deposition. Vitamin D deficiency has been associated with different components of the metabolic syndrome. Garanty-Bogacka et al. stated that hypovitaminosis D, common in obese adolescents at risk for type 2 diabetes (older age, puberty, Acanthosis nigricans), is associated with worse insulin resistance.\textsuperscript{34}

In a longitudinal study, vitamin D deficient children had an adjusted 0.1 per year greater change in BMI compared with vitamin D sufficient children (\(P\) for trend = 0.05). Similarly, vitamin D deficient children had a 0.03 per year (95% CI 0.01–0.05) greater change in the subscapular-to-triceps skinfold thickness ratio and a 0.8-cm/year (95% CI 0.1–1.6 cm/year) greater change in waist circumference compared with vitamin D sufficient children. These findings support the role of vitamin D in body fat deposition.\textsuperscript{35}

In the same direction of our results, Alemzadeh et al., Cheng et al.\textsuperscript{37} and Rajakumar et al.\textsuperscript{38} reported that the prevalence of vitamin D deficiency was 3-fold higher in those with high BMI and
adiposity. In Europe, Smotkin-Tangorra et al. also found an inverse correlation between 25(OH)D concentrations and BMI. There are contrary results for females with an increase in 25(OH)D concentrations according to BMI increase. Foo et al. reported similar results for Chinese adolescent girls. In contrast, other studies did not find any associations of BMI with 25(OH)D concentrations in the paediatric population. These controversial results with BMI led to consider not only the use of BMI but also the use of percentage of body fat and FFM to assess body composition in this population group. Our results reveal that FFM and upper limb strength positively correlate

Figure 1. 25(OH)D concentrations (nmol/L) by body composition in adolescents controlling by age, season and latitude.
with 25(OH)D concentrations in females. In agreement, Foo et al. observed same correlations in girls. Skeletal muscles require vitamin D for maximum function, with deficiency causing muscle weakness. In agreement with our results, positive relationship between vitamin D and muscle strength was recently observed in apparently healthy adolescent girls from the UK. Our data reinforce the observation made by Stewart et al., along with many other studies in children, providing compelling evidence that vitamin D is indeed, together with testosterone, the other steroid hormone that is important for muscle function and strength.
Our results show that vitamin D status is related not only with muscle strength but also with cardiovascular fitness. In males, cardiorespiratory fitness positive correlates with 25(OH)D concentrations. Inspection of the recent vitamin D epidemiological literature has brought to light the potential inverse relation of 25(OH)D concentrations with cardiovascular diseases.48 Similar to our results, in a recent study made in German adolescents, Lämmle et al.49 reported that lower 25(OH)D levels are associated with lower endurance performance and a higher BMI. Racinais et al.22 also found in apparently healthy adolescents from Qatar that the severely vitamin D deficient group had lower aerobic fitness than the moderately deficient group. In agreement, Zittermann et al.50 demonstrated an association between low vitamin D status and exercise intolerance.

These results together with those in the regression model suggest that physical fitness highly influences 25(OH)D concentrations in adolescents. This is one of the first studies dealing with objectively measured physical fitness tests rather than with physical activity (PA) questionnaires. These findings are of great interest for public health. The presence of abundant vitamin D receptors in myocardial tissue and vasculature and the observation that cardiovascular health may be ameliorated with sufficient vitamin D suggest a greater role of vitamin D in the cardiovascular system than expected.50

Regarding our results, a relationship between physical fitness and vitamin D concentrations is established. However, cross-sectional studies cannot determine the direction of this relationship, due to a higher vitamin D status could be caused by increased physical fitness and outdoor sun exposure (1) or a higher vitamin D status could lead to an increase in physical fitness (3).

Although we controlled for several potential confounders, data on testosterone were not available, and we cannot be certain that other unmeasured confounders have not influenced our observations. It should also be considered that sexual maturation was not included as a covariate in our analyses, due to the fact that most of the adolescents were at Tanner 4 and 5. However, the age of the adolescents was used instead, which showed a slight and stronger association with the dependent variables.

Despite the aforementioned limitation, the HELENA study has several strengths. The sampling procedure and the strict standardization of the field work 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 our study is the use of a large pool of variables, which provide a deeper understanding of the relationship between vitamin D status, body composition and physical fitness in European adolescents. The majority of the studies only assess PA and BMI but not fitness nor FFM and FM in relation to vitamin D, which is an additional strength of our work. The fitness tests used in the present report showed a good criterion-related validity in adolescents.27 In addition, this study includes important sets of confounders, i.e. age, seasonality or latitude which is crucial to analyse the association.

In conclusion, cardiorespiratory fitness is positively associated with 25(OH)D concentrations in male adolescents, whereas upper limbs muscular strength is positively associated with 25(OH)D concentrations in young females. In a similar way, higher FM in males and lower FFM in females are related to hypovitaminosis D. In both males and females, the interaction between fitness and BMI has a significant positive effect on 25(OH)D concentrations. Lifestyle and appropriate nutrition may prevent the long-term effect of hypovitaminosis D on health beyond mineral and bone metabolism. Therapeutic interventions to correct the high rates of vitamin D deficiency in adolescents should
consider muscular and cardiorespiratory physical fitness.

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Disclosure

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References


