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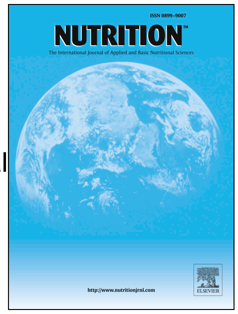

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25-hydroxyvitamin D is ~~differently~~ associated with calcium intakes of Northern, Central and Southern European adolescents: results from the HELENA study

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ABSTRACT

European (EU) adolescents exhibit a higher prevalence of vitamin D (VitD) deficiency than other age groups. The efficiency of sunlight exposure to increase 25(OH)D concentrations depends on a variety of factors, including diet. Nevertheless, the relationship between calcium and vitamin D (VitD) intake and 25 (OH)D concentrations have not been previously studied among adolescents living in different EU countries and consequently in different latitudes. Therefore, the aim of this study is to examine whether calcium and VitD intakes are differently associated with 25(OH)D in North, Central and South EU adolescents. 178 adolescents from Northern EU countries, 251 from Central EU countries and 212 from Southern EU countries aged 12.5-17.5 years were included in the current analyses. Mixed model linear regression analyses stratified by geographical location were used to verify associations between calcium and VitD intakes and

25(OH)D concentrations. Age, Tanner stage, seasonality, energy intake and supplement use were entered as covariates. Only calcium intakes of Central EU adolescents were positively associated with 25(OH)D (α= 0.005; CI 0.007, 0.028). Further longitudinal studies should confirm these observations, as this could be important for future public health interventions aiming to increase 25(OH)D concentrations among adolescents.

Keywords: latitude, vitamin D, status, Europe

INTRODUCTION

European (EU) adolescents exhibit a higher prevalence of vitamin D (VitD) deficiency than other age groups [1,2]. Such deficiency contributes to a higher risk of metabolic bone diseases and potentially other non-skeletal chronic diseases further in life. Hence, cost-effective public health VitD strategies are of great public health importance [1].

25-hydroxyvitamin D (25(OH)D) status is principally acquired through sunlight exposure, especially ultraviolet-B radiation (UV-B) activates the cutaneous synthesis of pre-vitamin D_3 in the skin [3]. The efficiency of sunlight exposure to increase 25(OH)D concentrations depends on a variety of factors; latitude, season, air pollution, sunscreen use, skin pigmentation, age, liver and kidney disease, and medication use, but the role of diet on 25 (OH)D status is still under debate [1].

VitD from dietary sources has been associated with 25(OH)D concentrations, especially during winter time. In adults, for every unit increase in VitD intake, 25(OH) D could increase by 1.0 nmol/L (summer/autumn) and 3.1 nmol/L (winter/spring) [4]. Calcium intake reduces circulating concentrations of calcitriol, which subsequently raises serum 25(OH)D concentrations and modulates the relationship between parathormone and 25(OH)D [5].

Nevertheless, the relationship between calcium and VitD intake and 25 (OH)D concentrations have not been previously studied among adolescents living in different EU countries and consequently in different latitudes. Therefore, the aim of this study is to examine whether calcium and VitD intakes are differently associated with 25(OH)D in North, Central and South EU adolescents.

MATERIAL AND METHODS

Study design

A subsample of 641 healthy adolescents (344 girls) aged 12.5-17.5 years from the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) who were not taking any medication, did not present any acute infection the week prior to the examination, provided data on two non-consecutive 24h dietary recalls and participated in the blood sampling were included for the purpose of this study. The sample included 178 adolescents from Northern EU cities (Dortmund in Germany and Stockholm in Sweden), 251 from Central EU cities (Ghent in Belgium, Lille in France and Vienna in Austria) and 212 from Southern EU cities (Athens and Heraklion in Greece, Rome in Italy and Zaragoza in Spain). The study was performed following the ethical guidelines of the Declaration of Helsinki 1964, the Good Clinical Practice rules and the legislation about clinical research in humans in each of the participating countries. The protocol was approved by the Human Research Review Committees of the institutions involved. All study participants and their parents provided a signed informed consent form.

Dietary assessment

Trained dieticians assisted adolescents to complete two non-consecutive 24h recalls, including weekdays and weekend-days. The 24h recalls were collected via the computer-based HELENA-Dietary Intake Assessment Tool (HELENA-

DIAT) [6]. The German Nutrient Database (BLS) was used to analyse the dietary data as it has been demonstrated good at estimating nutrient intake in European adolescents [7]. The Multiple Source Method (MSM) was used to estimate the usual dietary intake of nutrients and foods [8]. Adolescents were asked micronutrient supplement usage and were classified into two groups: supplement and non-supplement users.

Specimen collection and biochemical analyses

Fasting blood samples were collected by venipuncture at school between eight and ten o'clock in the morning between (October 2006 and June 2007) [9]. Blood was collected in EDTA tubes and transported at room temperature within 24 hours to the central IEL laboratory. Blood samples were centrifuged at 3500 rpm for 15 min at 4ºC and the supernatant stored at -80°C until assayed. The samples were stable for 24 hrs at room temperature (CV: 4.3%). Plasma 25-OH-Vitamin D_3 was analysed by ELISA using a kit (OCTEIA 25-Hydroxy Vitamin D) from Immunodiagnostic System and measured with a SunriseTM Photometer by TECAN (Germany). Blood sample date was used to compute seasonality defined as the following: winter (1; January through March), spring (2; April through June) and autumn (3; October through December). Summer was not included, because blood drawing was performed only during the academic year.

Statistical analysis

Analyses were carried out with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 20.0. Kolmogorov-Smirnov tests were carried out to test the normality of the data.

Mixed model linear regression analyses stratified by geographical location were used to verify associations between calcium and VitD intakes and 25(OH)D concentrations. Age, Tanner stage, seasonality, energy intake and supplement use were entered as covariates.

RESULTS

Table 1 shows descriptive characteristics of the participants. Average VitD intake and 25(OH)D concentrations were significantly different between Northern, Central and Southern EU adolescents ($p > 0.05$). Average calcium intake was not significantly different in Northern, Central and Southern EU adolescents ($p > 0.05$).

Table 2 presents the results of the mixed model linear regression analyses for calcium and VitD intakes and 25(OH)D concentrations. VitD intakes were not associated with 25(OH)D in the different geographical locations. Calcium intakes were positively associated with 25(OH)D ($\alpha= 0.005$; CI 0.007, 0.028) in Central EU adolescents ($p< 0.05$). No associations were observed in Northern and Southern EU adolescents for 25(OH)D and calcium intake.

DISCUSSION

Dietary intake is known to have a smaller contribution to 25(OH)D status [10] than cutaneous production in response to sun exposure. Nevertheless, we found a positive association between calcium intake and 25(OH)D status in Central EU adolescents. A previous study in Beijing adolescents who lived on similar latitudes, 46° N, as Central EU countries (mean of 50° N) showed positive associations between calcium intake and 25(OH)D status [11]. In our study, only calcium intakes of Central EU adolescents were associated with 25(OH)D. One hypothesis could be that those living on medium latitudes are those more dose-dependent on calcium dietary intakes due to the limited sunlight exposure in these Central EU cities (Lille, Ghent and Vienna). An increase in calcium intakes of 10mg/d was associated with an increase of 12nmol/l of 25(OH)D in our Central EU adolescents. This seems clinically relevant, because for each unit increase in milk (cup) or yogurt per day there was an average increase of 36nmol/l in 25(OH)D. Although this effect appears somewhat limited in absolute terms, it might still be important at the population level for the prevention of skeletal and non-skeletal chronic diseases further in life.

Results of Northern EU adolescents (Dortmund and Stockholm) are more difficult to interpret. Surprisingly we failed to find a statistically significant association between 25(OH)D and calcium intakes among Northern EU adolescents. Therefore, future studies with larger sample size should elaborate further on these preliminary results.

Limitations of this study should be taken into account. Results cannot be interpreted in terms of cause–effect relations, because of the cross-sectional design of the study. Adolescents were asked about taking any micronutrient supplements and supplementation was higher in Northern EU countries (data not shown), but the exact amount of calcium and VitD could not be calculated due to supplementation differences between countries. Nevertheless, we did not observe differences of calcium and Vit D dietary intakes between those adolescents taking supplementation or not.

CONCLUSIONS

Calcium intake seems to influence 25(OH)D status among Central EU adolescents. Further longitudinal studies should confirm these observations, as this could be important for future public health interventions aiming to increase 25(OH)D concentrations among adolescents.

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Table 1. Descriptive characteristics of Northern, Central and Southern EU adolescents (n=641)

	Northern EU adolescents (n= 178)	Central EU adolescents (n= 251)	South EU adolescents (n= 212)	P (ANOVA)*
Age (y)	14.6 ± 1.2	15.0 ± 1.1	14.5 ± 1.2	**<0.001**
Girls n (%)	81 (45.5)	146 (58.2)	117 (55.2)	**0.030**
Tanner stage				**<0.001**
Tanner I n (%)	0 (0)	2 (0.8)	3 (1.4)	
Tanner II n (%)	26 (15.0)	8 (3.2)	15 (7.1)	
Tanner III n (%)	68 (39.3)	25 (10.0)	56 (26.7)	
Tanner IV n (%)	74 (42.8)	133 (53.4)	83 (39.5)	
Tanner V n (%)	5 (2.9)	81 (32.5)	53 (25.2)	
Supplement users n (%)	38 (21.3)	27 (11.1)	15 (7.2)	**<0.001**
Energy intake (kcal/d)	2120.6 ± 764.5	2333.5 ± 907.4	2117.7 ± 693.9	**0.004**
25(OH)D (nmol/L)	53.0 ± 21.1	56.6 ± 26.6	69.1 ± 20.5	**<0.001**
Vit D intakes (µg/d)	1.8 ± 0.9	1.8 ± 0.9	2.2 ± 1.3	**<0.001**
Calcium intakes (mg/d)	870.8 ± 515.2	625.0 ± 582.4	867.3 ± 344.5	0.527

EU, European; 25(OH)D, 25-hydroxyvitamin D
Values are percentages for categorical variables; means ± SD for continuous variables
~~*~~Significant differences between Northern, Central and Southern European countries in bold ~~letters~~

Table 2. Mixed model analyses£ between 25(OH)D (nmol/L) with calcium (mg/d) and vitamin D (µg/d) intakes among EU adolescents

	25 (OH)D											
	North EU countries				Central EU countries				South EU countries			
	α	SE	95% CI	p	α	SE	95% CI	p	α	SE	95% CI	p
VitD intakes	2.222	2.215	-2.165, 6.609	0.318	1.627	1.780	-1.885, 5.139	0.362	-1.128	1.388	-3.869, 1.612	0.417
Calcium intakes	0.007	0.005	-0.002, 0.017	0.136	0.012	0.004	0.003, 0.020	**0.010**	-0.001	0.008	-0.016, 0.014	0.856

25(OH)D, 25-hydroxyvitamin D; EU, European
£adjusted for sex, age, Tanner stage, seasonality, energy intake and supplement use
~~*~~Significant values in bold ~~letters~~

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Highlights

1- Vitamin D intakes of European adolescents were not associated with 25-hydroxyvitamin D status
2- Only calcium intakes of Central European adolescents were positively associated with vitamin D status
3- Further longitudinal studies are needed to confirm the association between calcium intakes and vitamin D status considering different latitudes