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Determinants of vitamin D status in young children: results from the Belgian arm of the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) Study

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Abstract

Objective: To describe the vitamin D status of Belgian children and examine the influence of non-nutritional determinants, in particular of anthropometric variables.
Design: Cross-sectional data of Belgian participants of the EU 6th Framework Programme IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) Study.
Setting: 25-Hydroxyvitamin D (25(OH)D) was measured using RIA. Vitamin D status was categorized as deficient (<25 nmol/l), insufficient (25–50 nmol/l), sufficient (50–75 nmol/l) and optimal (≥75 nmol/l). Anthropometric measurements included height, weight, waist and hip circumferences and triceps and subscapular skinfold thicknesses.
Subjects: Children (*n* 357) aged 4–11 years.
Results: Serum 25(OH)D ranged from 13·6 to 123·5 nmol/l (mean 47·2 (SD 14·6) nmol/l); with 5 % deficient, 53 % insufficient, 40 % sufficient and 2 % optimal. No significant differences were found by age and gender. Significant differences in 25(OH)D were observed for month of sampling ($P < 0·001$), number of hours playing outside per week ($r = 0·140$), weight ($r = -0·121$), triceps ($r = -0·112$) and subscapular ($r = -0·119$) skinfold thickness, sum of two skinfold thicknesses ($r = -0·125$) and waist circumference ($r = -0·108$). Linear regression analysis of 25(OH)D adjusted for age, month of sampling and hours playing outside per week suggested that (i) weight, (ii) BMI *Z*-score, (iii) waist circumference and (iv) triceps and subscapular skinfold thickness (as well as the sum of both) independently influenced 25(OH)D.
Conclusions: The majority of Belgian children had a suboptimal vitamin D status, with more than half having an insufficient status in winter and spring. Month of the year, weekly number of hours playing outside and body composition – both central and abdominal obesity – were identified as important determinants of vitamin D status in Belgian children.

Keywords
Body composition
Children
Deficiency
Vitamin D

Vitamin D is an important determinant of bone health at all ages[1]. Vitamin D increases the absorption of Ca and phosphate from the gastrointestinal tract for mineralization of the skeleton. *In utero* and during childhood, vitamin D deficiency can cause growth retardation and skeletal deformities and is associated with an increased risk of hip fracture later in life[1–3]. In addition to its role in bone health, vitamin D has also been reported as a relevant factor in decreasing the risk of many chronic illnesses including common cancers and autoimmune, infectious and cardiovascular diseases[3]. Recently, Gilbert-Diamond *et al.*[4] demonstrated an inverse association between vitamin D serostatus and the development of adiposity in Colombian school-aged children.

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Vitamin D deficiency and insufficiency are still very common, especially in high-risk groups such as young children[3,5]. Humans get vitamin D from exposure to sunlight, from their diet and from dietary supplements. Previous studies showed that the dietary vitamin D intake of Belgian children and adolescents is very low compared with the Belgian recommendations[6,7]. However, no recent data on vitamin D status in young children living in Belgium are available. Moreover, most studies describing the vitamin D status of European children start at the age of 8 years and older[8–13]. Additionally, different anthropometric variables have been shown to significantly influence vitamin D status. Recently, Rodríguez-Rodríguez *et al.*[11] suggested that the amount of visceral and not subcutaneous fat determines the serum level of vitamin D in children (9–13 years old), but concluded that more studies are needed to test this hypothesis and to confirm their findings.

The study presented here describes cross-sectional data on the vitamin D status of Belgian children aged between 4 and 11 years, based on the serum concentration of 25-hydroxyvitamin D (25(OH)D) in blood samples taken in winter and spring, and aims to assess the influence of non-nutritional determinants on vitamin D status.

Experimental methods

Participants

Participating children were drawn from the Belgian control region cohort of the EU 6th Framework Programme IDEFICS Study, residents from the city of Aalter (51°05′N) in the northern Dutch-speaking part of Belgium. The IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS; http://www.idefics.eu) multicentre study is a European project in eight European countries. IDEFICS is a longitudinal study; however, in the present paper only the cross-sectional data collected in 2010 are used. The children were contacted by random cluster sampling (all children from a selection of classes from all schools in the control city)[14–16]. During the fieldwork period, which ran from February 2010 to June 2010, a venous blood collection was received from 509 children in the Belgian IDEFICS control region. Vitamin D analysis was performed on the samples of 358 children. Blood drawn from the rest of the children was not sufficient to allow analysis, since the first six aliquots were analysed for other purposes of the IDEFICS Study. For one child, information on sex was missing. Therefore, the data of 357 children were used for the purposes of the present analysis. Concerning the ethnicity of the children, of the 357, only seven were not born in Belgium (four of the seven were born within the EU). For the children who were born in Belgium, only two had one parent born outside Belgium (one from Cuba and one from the Philippines). The study was conducted according to the guidelines laid down in the Helsinki Declaration of the World Medical Association and the project protocol was approved by the Ethical Committee of Ghent University Hospital. All parents of the participating children gave written informed consent.

Questionnaire

A self-administered parental questionnaire was used to obtain information on the following variables: number of hours playing outside during weekdays and weekend days, use of vitamin supplements, birth weight and fractures. Questions assessing the number of hours playing outside were based on those used in Burdette *et al.*[17].

Anthropometric measurements and body composition

All anthropometric measurements were done by two trained researchers. Height and weight were measured respectively with a standard clinical Seca 225 stadiometer (Seca GmbH & Co. KG, Hamburg, Germany) to the nearest 0·1 cm and a balance (Tanita BC 420 SMA; Tanita, Amsterdam, The Netherlands) to the nearest 0·1 kg, without shoes and in light clothing. The Tanita balance was calibrated and did not need further calibration; also the Seca stadiometer needed neither maintenance nor further calibration. BMI was calculated according to the formula: BMI = weight (kg)/[height (m)]^2. For each child, weight, height and BMI *Z*-score and the International Obesity Taskforce grade was determined using the LMS method (with British reference population) which summarizes the distribution of weight, height and BMI at each age by its median and CV, plus a measure of skewness based on the Box–Cox power required to transform the data to normality[18]. Waist and hip circumferences were measured using a Seca 200 inelastic tape (Seca GmbH & Co. KG; precision 0·1 cm, range 0–150 cm). This tape did not need any calibration. Skinfold thickness was measured at the previously marked points using Holtain Tanner/Whitehouse skinfold callipers (Holtain Ltd, Crosswell, UK; range 0–40 mm). The callipers were calibrated every morning and additionally when dropped by means of a calibration block of 20 mm. Skinfold thickness was measured at two sites (triceps and subscapular) according to the international standards for anthropometric assessment (International Society for the Advancement of Kinanthropometry)[19]. Skinfold thickness was measured twice at each site and the mean of both measurements was calculated. For the different body composition parameters, mean *Z*-scores were calculated using the formula: $Z = (X - \mu)/\text{SD}$, with X the measured value, μ the mean and SD the standard deviation of the parameter.

Biochemical analysis

Fasting blood samples (10 ml) were drawn by venepuncture by a medical doctor. The serum was separated, aliquoted and stored at −80°C until analysis. 25(OH)D concentrations were measured by RIA (DiaSorin 25(OH)D – ^{125}I RIA kit, Stillwater, MN, USA). The inter-assay CV for low and higher 25(OH)D controls was 6·2% and 6·5%, respectively, whereas the intra-assay CV was 8·4% and 7·3% for low and higher 25(OH) controls, respectively. For validation of the vitamin D assay, the laboratory participates in the External Quality Assessment Scheme of SKML (Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek) from The Netherlands, which six times a year gives two samples of vitamin D controls.

Statistical analysis

Pearson correlation coefficients were calculated to investigate correlations between vitamin D status and other continuous variables. Differences in vitamin D status between two groups (sex, (non-)use of vitamin D supplements and (not) having had fractures in the past) were examined by the independent-samples *t* test. When more than two groups were considered, ANOVA was used. In addition, as exploratory analysis, the *post hoc* Tukey Honestly Significant Difference (HSD) test was performed to make pairwise comparisons.

The independent contribution of non-nutritional determinants (age, gender, month of sampling, number of hours playing outside per week) to the variance in vitamin D status was examined by analysis of covariance (ANCOVA). Two ANCOVA models were constructed using two different indicators of body composition: (i) the sum of two skinfold thicknesses, reflecting the amount of subcutaneous fat; and (ii) waist circumference, reflecting the amount of abdominal fat. Both body composition parameters were log-transformed to obtain normality in these variables. Only significant variables associated with vitamin D status were included in the final model. The effect of clustering was investigated using mixed model analyses, with 'school' being the nested variable and taking on board the same covariates and interaction factors as was done in the ANCOVA model.

Relationships between vitamin D status and related anthropometric variables (weight, height, BMI *Z*-score, waist circumference, waist:hip ratio, waist:height ratio, triceps skinfold thickness, subscapular skinfold thickness, sum of two skinfold thicknesses) were quantified using linear regression, controlling for age, month of sampling and number of hours playing outside per week. For these analyses, the month of sampling was dummy coded (March and April = 0; May and June = 1; the cases of February (n 29) were excluded).

All analyses were performed using the SPSS for Windows statistical software package version 15·0 (SPSS Inc., Chicago, IL, USA), and values of $P < 0·05$ were considered statistically significant.

Results

Characteristics and anthropometric variables of the population sample are summarized in Table 1. Serum 25(OH)D ranged from 13·6 nmol/l to 123·5 nmol/l, with a mean concentration of 47·2 (SD 14·6) nmol/l.

No significant difference in vitamin D status was found between boys and girls ($P = 0·263$). Children using

Table 1 Characteristics, serum 25(OH)D concentrations and anthropometric data of the study population: Belgian children aged 4–11 years participating in the IDEFICS Study, 2010

	Study sample (n 357)		
Variable	Mean or %	SD	*Z*-score
Age (years)	8·1	1·5	
Gender: male (%)	51·5		
Born in Belgium (%)	98·0		
Serum 25(OH)D concentration (nmol/l)	47·2	14·6	
Serum 25(OH)D concentration <25 nmol/l (%)	5		
Serum 25(OH)D concentration between 25 and 50 nmol/l (%)	53		
Serum 25(OH)D concentration between 50 and 75 nmol/l (%)	40		
Serum 25(OH)D concentration >75 nmol/l (%)	2		
Number of hours playing outside (h/week)	14·0	8·5	
Vitamin D supplement: yes (%)	5·9		
Fracture: yes (%)	12·6		
Weight (kg)	27·0	5·7	0·078
Height (cm)	130·0	10·1	0·456
BMI (kg/m^2)	15·8	0·1	−0·267
BMI (%)			
Underweight	13·4		
Normal weight	81·5		
Overweight	5·0		
Triceps skinfold thickness (mm)	10·2	3·5	0·018
Subscapular skinfold thickness (mm)	6·4	2·8	0·007
Waist circumference (cm)	56·3	5·4	0·032
Waist:hip ratio	0·85	0·12	−0·002
Waist:height ratio	0·43	0·04	0·117

25(OH)D, 25-hydroxyvitamin D; IDEFICS, Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants.

vitamin D-containing supplements (5·9 %) had a mean 25(OH)D concentration of 49·5 (SD 11·2) nmol/l compared with 47·0 (SD 15·2) nmol/l for those not using vitamin D-containing supplements; however, the difference was not significant ($P = 0·464$). On the same line, no significant difference ($P = 0·302$) was found between those children who reported having had a fracture (12·6 %) so far (25(OH)D = 44·9 (SD 13·7) nmol/l) and those without any reported fractures (25(OH)D = 47·3 (SD 13·7) nmol/l).

ANOVA did not show any significant difference in 25(OH)D concentration for different birth months ($P = 0·160$). However, a significant difference in 25(OH)D concentration between the months of sampling (February to June) was found ($P < 0·001$). The *post hoc* Tukey HSD test indicated a difference between March and May ($P < 0·001$), March and June ($P < 0·001$), April and May ($P = 0·007$) and April and June ($P = 0·002$), as illustrated in Fig. 1.

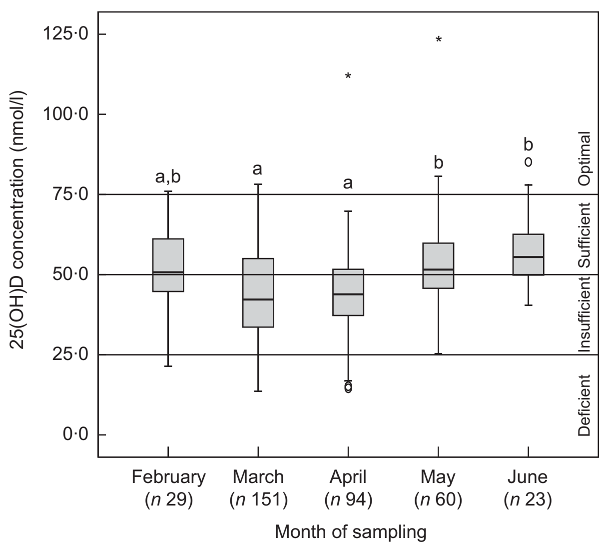

Fig. 1 Box plot of serum 25-hydroxyvitamin D (25(OH)D) concentrations in Belgian children aged 4–11 years participating in the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) Study, 2010, according to month of sampling. [a,b]Mean values with unlike superscript letters were significantly different ($P < 0·05$); ○, outlier; *, extreme value

Table 2 shows the correlation coefficients between 25(OH)D concentration and several covariates of interest. A positive significant correlation ($r = 0·140$) was found between 25(OH)D concentration and the number of hours playing outside per week. Moreover, negative correlations were found between 25(OH)D concentration and weight ($r = -0·121$), triceps ($r = -0·112$) and subscapular ($r = -0·119$) skinfold thickness, the sum of two skinfold thicknesses ($r = -0·125$) and waist circumference ($r = -0·108$).

The results of the ANCOVA models are shown in Table 3. The adjusted R^2 (coefficient of determination) of the first (with skinfold thickness) and second (with waist circumference) model was 0·126 and 0·118, respectively. Both models indicated a significant contribution of the month of sampling, the number of hours playing outside per week and the included body composition parameter (reflecting respectively subcutaneous and abdominal fat) to the variance in 25(OH)D concentration.

The mixed model analyses indicated that the cluster random sampling design of the study (via schools) did not have an influence on the study results ($P = 0·198$).

Using linear regression analyses with serum 25(OH)D concentration as the dependent variable and controlling for age, month of sampling and number of hours playing outside per week, it was found that weight, BMI *Z*-score, waist circumference and triceps and subscapular skinfold thickness (as well as the sum of both) independently influenced these concentrations (Table 4).

Discussion

The present paper describes the vitamin D status of Belgian children aged 4–11 years. The optimal vitamin D levels for health remain a subject of debate[3]. In the present paper four categories were considered: category 1, <25 nmol/l, deficient; category 2, 25–50 nmol/l, insufficient;

Table 2 Pearson correlation coefficients between 25(OH)D concentration and several covariates: Belgian children aged 4–11 years participating in the IDEFICS Study, 2010

	Pearson correlation coefficient	*P* value
Age (years)	−0·030	0·578
Number of hours playing outside per week	0·140	0·011*
Weight (kg)	−0·121	0·022*
Height (cm)	−0·070	0·188
BMI *Z*-score	−0·080	0·131
Triceps skinfold thickness (mm)	−0·112	0·034*
Subscapular skinfold thickness (mm)	−0·119	0·025*
Sum of triceps and subscapular skinfold thickness (cm)	−0·125	0·018*
Waist (cm)	−0·108	0·042*
Waist:hip ratio	−0·097	0·068
Waist:height ratio	−0·038	0·472

25(OH)D, 25-hydroxyvitamin D; IDEFICS, Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants.
*Significant at level $P = 0·05$.

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Table 3 Results of the two ANCOVA models assessing the independent contributions of determinants to the variance in 25(OH)D concentration: Belgian children aged 4–11 years participating in the IDEFICS Study, 2010

	F factor	*P* value
First model (adjusted $R^2 = 0{\cdot}126$)		
Month of sampling	3·4	0·009*
Interaction between month of sampling and hours playing outside per week	3·2	0·007*
Sum of triceps and subscapular skinfold thickness	8·2	0·004*
Second model (adjusted $R^2 = 0{\cdot}118$)		
Month of sampling	3·2	0·014*
Interaction between month of sampling and hours playing outside per week	2·9	0·013*
Waist circumference	6·7	0·010*

ANCOVA, analysis of covariance; 25(OH)D, 25-hydroxyvitamin D; IDEFICS, Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants.
*Significant at level $P = 0{\cdot}05$.

Table 4 Regression results using 25(OH)D concentration as the dependent variable†: Belgian children aged 4–11 years participating in the IDEFICS Study, 2010

	Standardized coefficient β value	*P* value
Weight (kg)	−0·234	0·001*
Height (cm)	−0·188	0·053
BMI *Z*-score (kg/m^2)	−0·119	0·029*
Waist circumference (cm)	−0·153	0·010*
Waist:hip ratio	−0·090	0·121
Waist:height ratio	−0·094	0·107
Triceps skinfold thickness (mm)	−0·163	0·012*
Subscapular skinfold thickness (mm)	−0·140	0·010*
Sum of two skinfold thicknesses (mm)	−0·153	0·005*

25(OH)D, 25-hydroxyvitamin D; IDEFICS, Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants.
*Significant at level $P = 0{\cdot}05$.
†Confounding variables (age, month of sampling and number of hours playing outside per week) have been taken into account in the statistical analysis.

category 3, 50–75 nmol/l, sufficient; and category 4, ≥75 nmol/l, optimal. These categories are based on recent publications taking into account the full advantage of all the health benefits that vitamin D provides[3,5,20]. More specifically for children, the Pediatric Endocrine Society stated that 50 nmol/l is the lower end of the optimal 25(OH)D concentration in children[21]. Within our study sample of Belgian children, 58 % had an insufficient vitamin D status and only a small proportion (2·2 %) reached the optimal level, based on blood sampled in winter and spring. Until now, recent data on the vitamin D status of Belgian young children had not been available. Evidence on the vitamin D status of other European children up to 10 years is limited as well, most including an older age group.

Comparison with other European data

The data of the present study were compared with other available European data; however, this comparison must be interpreted with caution, as not all the studies used the same method of determining 25(OH)D which can influence the results. At the end of the last millennium, data were published on the vitamin D status of French, Spanish and Finnish children aged 13–17 years, 8–12 years and 9–15 years, respectively[8–10]. In the French children (only males), a mean 25(OH)D concentration of 58·5 nmol/l was found after summer and 20·6 nmol/l was found after winter[10]. Similarly, in the Spanish children, a mean of 74·8 and 31·5 nmol/l was determined in October and March, respectively[9]. This illustrates a strong effect of the season of sampling, similarly to that observed in our study (e.g. March *v.* June; Fig. 1). The Finnish study, which included only girls, indicated a mean 25(OH)D concentration of 33·9 nmol/l[8], lower than the mean found in the present study. More recent publications describing the vitamin D status in European children, in Spain (9–13 years)[11], in Northern Ireland (12–15 years)[12] and in a combined cohort of Finnish and Danish girls (11 years old)[13], indicated higher concentrations compared with our Belgian sample. For instance, mean 25(OH)D concentrations in Spain were 49·6 nmol/l[11], in Northern Ireland were 56·7 nmol/l (winter) and 78·1 nmol/l (summer), in the Danish and Finnish girls were 57·2 and 56·2 nmol/l, respectively[13], as opposed to 47·2 nmol/l in our sample. We found no significant differences by gender, unlike the Spanish and Northern Irish populations where significant differences by gender were observed. However our study population was younger and this age difference could have an influence on gender differences.

Determinants of vitamin D status

The ANCOVA yielded three important non-nutritional determinants of the vitamin D status in Belgian young

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children: month of sampling, number of hours playing outside per week and body composition. The first two parameters are related to the fact that solar UV-B radiation converts and modifies 7-dehydrocholesterol into vitamin D at skin temperature[2]. In northern countries, such as Belgium, there is no UV radiation of the appropriate wavelength from the end of October to the end of March[22]. This is clearly illustrated in Fig. 1, showing a minimum in 25(OH)D in March and an increase from April on. Moreover, the study results showed an interaction effect of season and the weekly number of hours playing outside, since the highest levels of outdoor playtime occur in summer and the lowest in winter[17].

Body composition is another important determinant of vitamin D status. Previous studies have suggested that vitamin D deficiency is associated with excess body weight mainly due to the fact that body fat could function as a sink for vitamin D, which is fat soluble[23–25]. Moreover, inadequate vitamin D status could also be a risk factor for childhood obesity, since it affects lipolysis and adipogenesis in human adipocytes[26–29]. In the present study, it was found that parameters of whole-body obesity (defined by the BMI), parameters of subcutaneous fat (skinfold thickness) as well as parameters of abdominal fat (waist circumference) independently influenced vitamin D status in young Belgian children (4–11 years old). The study of Spanish children (9–13 years)[11] also concluded that BMI and abdominal obesity influenced the appearance of vitamin D insufficiency in children. However, the Spanish study did not find a difference in terms of the amount of subcutaneous fat between the children with insufficient vitamin D levels and the children with adequate levels. The difference concerning this finding between both studies can possibly be influenced by the difference in age (on average 8·1 years in Belgium *v.* 10·9 years in Spain), resulting in lower weight, height, BMI, triceps skinfold thickness and waist circumference in Belgium compared with Spain.

Strengths and limitations

The availability of various anthropometric parameters measured in a standardized way in a relatively large sample is one of the strengths of the present study. In this way the authors had the opportunity to address the influence of various anthropometric indicators on vitamin D status and explore possible relationships and interactions. The present study is one of the first addressing this issue in young children, based on the hypothesis suggested by Rodriguez-Rodriguez *et al.*[11]. To assess vitamin D status, the serum concentration of 25(OH)D was measured, currently the best parameter for vitamin D status[5,20]. Next, the questions assessing the number of hours playing outside were based on Burdette *et al.*[17], concluding that parent-reported measures of outdoor playtime were significantly correlated to a direct measure of physical activity in children of pre-school age and are worthy of future evaluation as a survey measure. Although the analyses controlled for several potential confounders we cannot be certain that other unmeasured confounders such as genetic variation or dietary intake have not influenced our observations. Nevertheless, concerning dietary intake, the number of food items on the Belgian market that are fortified with vitamin D is rather limited, i.e. margarine (mandatory fortified), growth milk and some normal milks as well as breakfast cereals (depending on the brand). More detailed figures about the dietary intake of vitamin D in Belgian pre-school children have recently been published[30].

Conclusions

The majority of Belgian children (4–11 years old) have a suboptimal vitamin D status, with more than half having an insufficient status during winter and spring months. Month of the year, number of hours playing outside per week as well as body composition were identified as important determinants of vitamin D status in this group of children. The vitamin D status of these children was independently influenced by parameters of whole-body obesity, parameters of subcutaneous fat as well as parameters of abdominal fat. The impact of recommendations to optimize the vitamin D status of young children should be investigated in order to prevent associated diseases.

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