Research Article

Association between vitamin D status and obesity in Bulgarian pre-pubertal children: a pilot study

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ABSTRACT

Background: It is considered that obesity and metabolic syndrome are accompanied with vitamin D deficiency. We aimed to examine the interrelations between vitamin D status and biomarkers for metabolic syndrome in Bulgarian pre-pubertal children.
Methods: The study enrolled 51 pre-pubertal children (29 boys, 22 girls) examined for serum 25-xydroxyvitamin D, and routine parameters for metabolic syndrome. Obesity was evaluated by body mass index and waist circumference.
Results: More than half (57.1%) of the studied children were vitamin D deficient, prevalent in girls than in boys (65.0% vs. 51.7% respectively). A tendency for worse metabolic status in the vitamin D-deficient group, expressed by higher fasting insulin, total cholesterol, total cholesterol/HDL-ratio and Homeostasis Model Assessment (HOMA)-index was observed. A trend for negative correlation was established between 25-xydroxyvitamin D and waist circumference, HOMA-index, and fasting insulin.
Conclusions: Vitamin D deficiency and inverse relationships between 25-xydroxyvitamin D and waist circumference, HOMA-index, and insulin were found amongst studied children.

Keywords: 25-hydroxyvitamin D, Pre-pubertal children, Obesity, Insulin resistance

INTRODUCTION

It is considered that there is a relationship between the risk of chronic disease development in adult age and vitamin D deficiency during childhood.[1] Data recorded in the past years shows that 20-42% of healthy children aged 11-18 years are vitamin D deficient.[2]

It is widely accepted that the best indicator for assessment of vitamin D status is the circulating form of cholecalciferol - 25-xydroxyvitamin D (25OHD). According to the American Endocrinology Society the serum circulating 25-hydroxyvitamin D level should be used to evaluate high-risk individuals for deficiency, which is defined as a 25OHD <50 nmol/L. Deficiency also may include insufficiency, which is defined as 25OHD of 50 nmol/L to 72.5 nmol/L.[3]

The extensive NHANES research, conducted between 2001-2004 by the Centre for Control and Disease Prevention in the USA, involving about 10000 children (1-21 years), indicates that 9% of them have severe vitamin D deficiency (under 37.5nmol/L), and 61% display levels between 37.5 and 72.5nmol/L.[4] A study on vitamin D status for children in the preadolescent age, carried out in the USA, reveals that 48% of the residents, living at the middle latitude of 44o are vitamin D deficient (<50nmol/L) all year round. Those from more southern regions, with middle latitude of 32°, experience vitamin D deficiency only during the winter season.[5]

Similar studies, undertaken in Europe, confirm the high percentage of vitamin D deficiency amongst the child population.[6] Bulgaria is a South European country with main latitude 43° North. Therefore, it is very likely to expect similar rate of vitamin D deficiency amongst Bulgarian children in this age, as well. Beside the geographic location of Bulgaria, another prerequisite to expect high rate of vitamin D deficiency in children is recently proved high rate of vitamin D deficiency in adults.[7]

Vitamin D synthesis starts in the skin and dependents on sun exposure, specifically UVB radiation. Ordinarily, vitamin D deficiency is related to inadequate dietary intake and insufficient sun exposure. The widespread sunscreen use decreases the dermal biosynthesis of vitamin D and is considered as a reason for vitamin D deficiency. An additional factor for decreased bioavailability of vitamin D can be its increased uptake in the adipose tissue. There are publications, verifying the negative correlation between serum levels of 25OHD and accumulated adipose tissue for children with obesity.[8,9] It is suggested, that the fat-soluble vitamin D is absorbed in the subcutaneous adipose tissue, leading to decreased plasma 25OHD levels. Henceforth, the remaining tissues are vitamin D insufficient.[10,11] The frequently observed increased levels of PTH in children with high body mass index (BMI), could be a result of vitamin D deficiency.[8,12] There is a hypothesis suggesting that increased PTH boosts the intracellular calcium in adipocytes, which stimulates the lipogenesis and leads to additional weight gain.[13]

Consequently, a causal vicious cycle is being created, linking vitamin D deficiency, PTH and obesity. Therefore the interrelation between vitamin D deficiency and indexes evaluating metabolic syndrome – increased BMI, blood pressure, impaired glucose tolerance, reduced HDL-cholesterol is intensively studied in obese children.[2,14]

There is a limited number of studies on vitamin D status in pre-pubertal children, and its relation to obesity and prognostic biomarkers for metabolic risk.

Despite the increased occurrence of overweight and obese children in young age[15], studies in Bulgaria about the prevalence of vitamin D deficiency for this particular age group are lacking. We aimed to perform a pilot research on vitamin D status in normal and overweight pre-pubertal children evaluating its seasonality and interrelations with anthropometric indexes and biomarkers related to the metabolic risk.

METHODS

The present pilot study enrolled 51 consecutive healthy pre-pubertal children, the parents/guardians of whom gave written informed consent, 29 males (56.9%) and 22 females (43.1%) from Varna region who were investigated at the First Pediatric Clinic at the University Hospital of Varna. The age of the studied group was 4.0-8.0 years (boys: 4.75-11.0 years; girls: 4.0-10.0 years). All participants were pre-pubertal (Tanner stage I). Sixteen children with normal weight (BMI<85-th percentile), and 35 children with overweight/obesity (BMI>95-th percentile), according to the IOTF reference for BMI were included in the study.[16] Subjects with pre-existing chronic diseases of calcium-phosphate metabolism or other diseases affecting calcium metabolism, chronic inflammatory disorders, and infectious diseases were excluded from the study. The usage of vitamin D, calcium or phosphorus medications during the last 6 months prior the study was another exclusion criterion. All children were healthy at the time of investigation. The required information was obtained by a parental structured interview and full physical examination of the participants.

Auxological and Blood pressure measurements. All anthropometric measures were taken by the same well-trained investigator with children wearing light clothing and no shoes. Weight was measured to the nearest 0.1 kg using a calibrated digital scale (TANITA Ltd., Middlesex, UK). Height was measured to the nearest 1 mm with a portable stadiometer (Seca Ltd., Hamburg, Germany), with the child upright and the head in the Frankfurt plane. WC was measured to the nearest 1 mm with a flexible, non-elastic tape midway between the tenth rib and the iliac crest at the end of a gentle expiration. BMI was calculated as weight (kg)/height^2 (m^2). Pubertal development was clinically assessed based on secondary sexual characteristics according to Tanner's criteria. None of the subjects had begun puberty. Blood pressure (BP) was measured using a mercury sphygmomanometer KaWeM5 (Kirchner&Wilhelm GmbH+Co. KG, Asperg, Germany) according to the recommendations of the National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Children and Adolescents.[17]

Biochemical tests

Fasting blood samples were collected and transferred to the Clinical laboratory for analysing the routine laboratory parameters. Fasting blood glucose (FBG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were measured by homogeneous colorimetric enzyme technique. FBG was measured by enzyme (hexokinase) UV technique. Routine colorimetric method was used for quantitation of serum calcium (Ca) levels. C-reactive protein (CRP) was determined by turbidimethric method. All these parameters were measured on ADVIA®1800 Clinical Chemistry System (Siemens Healthcare Diagnostics, USA). Chemiluminiscent immunometric methods and Immulite 2000 Immunoassay System (Siemens Healthcare Diagnostics, USA) were used for measuring fasting blood insulin (FBI) and intact parathyroid hormone (iPTH).

Insulin resistance (IR) was estimated by the homeostasis model assessment (HOMA).

25OHD assay

Serum samples for measuring 25OHD were collected only once – at the time of their admittance to the Pediatric Clinic. All serum samples were frozen, and stored at -80°C until analysis. Serum 25OHD was assayed by a validated HPLC-UV method.[18] The vitamin D status was defined as severe deficiency (25OHD<25nmol/L), deficiency (25OHD<50nmol/L), sufficiency (25OHD>50nmol/L).

As this is not a follow-up study all parameters were measured only once.

Statistical analysis

Categorical variables were presented as frequencies (%). GraphPad Prism v. 6.00 for Windows (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis of continued variables. One way ANOVA with Bonferoni's multiple comparison post-test was used for comparison of means of different parameters. Mann Whitney t-test was performed to evaluate the differences between medians of two unmatched groups. The level of significance was $P<0.05$. The Pearson correlation coefficient (r) was used to determine the degree of linear relationships between 25OHD and other tested parameters.

RESULTS

Table 1: Demographic, anthropometric, and biochemical parameters of the studied group.

Parameters	Value (mean±SD)	Parameters	Value (mean±SD)
Gender		Age, years	7.99±1.82
Boys, n/N (%)	29/51 (56.9%)	Boys	8.58±1.60
Girls, n/N (%)	22/51 (43.1%)	Girls	7.00±1.72
Weight, kg	39.39±14.17	Height, cm	130.00±14.48
Boys	44.04±14.41	Boys	134.6±13.93
Girls	33.25±11.48	Girls	124.1±13.22
BMI, kg/m^2	22.50±5.26	WC, cm	77.70±11.17
Boys	23.54±5.03	Boys	81.95±9.88
Girls	21.13±5.36	Girls	71.73±10.29
Normal, (%)	16.05±2.22, (31.40%)	Normal, (%)	65.48±5.90, (30.53%)
Boys	17.82±3.29, (37.26%)	Boys	69.70±5.52, (19.60%)
Girls	16.37±2.53, (19.60%)	Girls	61.86±3.34, (21.56%)
Overweight + obese, (%)	25.45±3.17, (68.60%)	Overweight/obese, (%)	82.95±8.41, (69.77%)
Boys	26.55±2.51, (19.60%)	Boys	85.82±7.47, (34.26%)
Girls	25.10±3.44, (23.53%)	Girls	78.01±7.86, (21.56%)
SBP, mmHg	104.60±12.00	DBP, mm Hg	66.48±9.79
Boys	105.9±14.14	Boys	67.0±10.73
Girls	102.8±8.47	Girls	65.79±8.71
FBG, mmol/L	5.10±0.81	FBI, µU/ml	14.31±8.25
Boys	5.11±0.82	Boys	13.66±8.47
Girls	5.09±0.81	Girls	15.15±8.14
HOMA-index	3.43±2.25	TC/HDL ratio	4.24±6.22
Boys	3.22±2.32	Boys	3.50±0.75
Girls	3.70±2.19	Girls	5.85±9.10
TG, mmol/L	0.97±0.39	TC, mmol/L	4.82±4.74
Boys	1.05±0.37	Boys	4.31±1.01
Girls	0.88±0.40	Girls	5.46±7.10
HDL-C, mmol/L	1.26±0.26	LDL-C, mmol/L	2.43±0.85
Boys	1.26±0.17	Boys	2.58±0.92
Girls	1.27±0.34	Girls	2.28±0.76
CRP, mg/L	2.73±2.48	Calcium, mmol/L	2.49±0.10
Boys	2.51±2.37	Boys	2.46±0.09
Girls	3.0±2.65	Girls	2.52±0.14
iPTH, ng/L	26.83±26.57		
Boys	25.36±17.94		
Girls	28.68±35.26		

[1] BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood pressure; FBG – fasting blood glucose; FBI – fasting blood insulin; TG – triglycerides; TC – total cholesterol; CRP – C-reactive protein; iPTH – intact parathyroid hormone.

The means and SD were calculated by descriptive analysis. N, Total number of studied children = 51 (Table 1).

Demographic, anthropometric, and biochemical parameters are shown in Table 1.

The mean 25OHD levels of the studied group (49.65±19.82nmol/L) almost approach the cut-off value of 50nmol/L, which defines vitamin D sufficiency. Forty nine children were tested for 25OHD levels and stratified by their vitamin D status. Twenty one subjects of them (42.9%) were vitamin D sufficient, showing values above 50nmol/L; 28 subjects (57.1%) were vitamin D deficient with 25OHD<50nmol/L; 4 children (8.2%) were severely deficient with 25OHD<25nmol/L. Only 6 children (12.2%) reached 25OHD levels above the desirable threshold of 75nmol/L. After categorization by gender 48.3% of boys and 35.0% of girls were vitamin D sufficient. Values above the threshold of 75nmol/L were found in 13.79% of boys and in 10.0% of girls. Vitamin D deficiency was detected in 51.7% of pre-pubertal boys and in 65.0% of the girls. One boy (3.4%) and 3 girls (15.0%) were with severe vitamin D deficiency, showing values of 16.94nmol/L and 18.83±6.06nmol/L, respectively. There was no statistical significance in vitamin D status between boys and girls (Table 2).

Table 2: Stratification of the studied group by the serum 25OHD levels.

Vitamin D status	Total studied group mean±SD, (n)	Pre-pubertal boys mean±SD, (n)	Pre-pubertal girls mean±SD, (n)
Severe deficiency (25OHD<25nmol/L)	18.36±5.04, (4)	16.94, (1)	18.83±6.06, (3)
Deficiency (25OHD<50nmol/L)	38.20±11.51, (28)	39.03±9.047, (15)	33.83±10.94, (13)
Sufficiency (25OHD>50nmol/L)	67.74±16.43, (21)	67.56±17.29, (14)	65.99±13.42, (7)
Desired threshold (25OHD>75nmol/L)	88.33±11.50, (6)	90.16±13.87, (4)	84.68±6.67, (2)

1 25OHD – 25-hydroxy vitamin D. The data for 25OHD are given in nmol/L

Table 3: Seasonality of serum 25OHD levels.

Season	Total study group mean±SD, (n)	Pre-pubertal boys mean±SD, (n)	Pre-pubertal girls mean±SD, (n)
Winter	45.74±17.23, (36)	49.98±16.91, (22)	39.09±16.12, (14)
Summer	60.48 ±23.07, (13)	61.68±26.45, (7)	59.09±20.81, (6)

Since the 25OHD levels show considerable seasonality, we stratified the studied population by the season of blood drawing as follows: winter (November-March), and summer (April-July). Thirty six subjects (73.5%) were assayed during winter and 13 (26.5%) in the summer. There were significant differences between the winter and summer mean 25OHD levels (P<0.05) (Table 3). During the summer the 25OHD levels for both genders were in the range of vitamin D sufficiency, while for the winter season vitamin D deficiency was detected for both groups. Twenty eight percent higher values for males were detected during winter compared to females (P=0.06). During summer the mean values for both genders are almost the same.
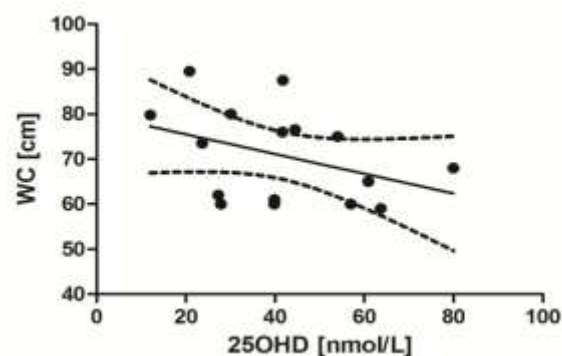

Figure 1: Negative relationship between 25OHD levels and waist circumference (WC) in pre-pubertal girls (Pearson r=-0.38, P>0.05).

Next we stratified the studied subjects into vitamin D deficient (25OHD<50nmol/L) and vitamin D sufficient (25OHD>50nmol/L) groups. The differences in 25OHD levels between these groups, 67.74±16.43 vs. 36.62±10.13 nmol/L, were found to be statistically significant (P<0.001). Lower WC values were detected for the vitamin D sufficient group (75.27±9.81 cm), and higher WC for vitamin D deficient children

(80.20±7.73cm). The difference between both groups reached a borderline value for statistical significance P=0.055. Examining the relationship between 25OHD and WC, we established a trend for negative correlation: Pearson r=-0.38, P>0.05 (Figure 1). Surprisingly the BMI values did not differ significantly between these two groups. Tendency for worsened metabolic parameters in the vitamin D deficient group were expressed by higher FBI (p=0.13) and HOMA-index (p=0.08). There were no differences between the groups for systolic or diastolic blood pressure FGB, TG, TC, HDL-C, LDL-C, TC/HDL-C, and Ca levels.

Not surprisingly we found a significant negative correlation between 25OHD levels and PTH for the entire studied group, Pearson r=-0.38, P=0.03 (Figure 2). This negative relationship was more strongly expressed in the group of pre-pubertal girls (Pearson r = -0.60, P=0.018).
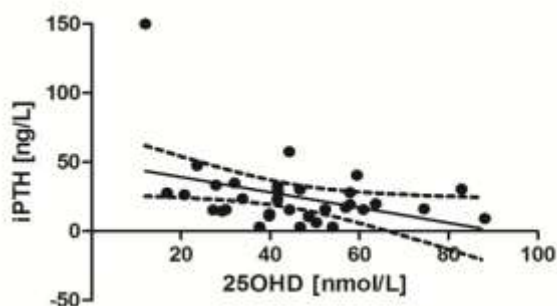

Figure 2: Negative association between 25OHD levels and iPTH for the studied pre-pubertal children (Pearson r=-0.38, P=0.03).

Serum 25OHD levels were inversely associated with HOMA-index and FBI levels for the entire studied group (Figure 3). The same non-significant tendency for both parameters was observed when the participants were stratified by gender. For the association HOMA/25OHD the Pearson r was -0.24 and -0.25 for pre-pubertal boys and girls, respectively; for the association FBI/25OHD the Pearson r was -0.27 and -0.21 for pre-pubertal boys and girls.
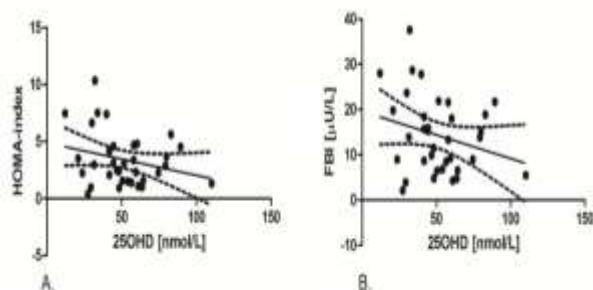

Figure 3: Negative association between 25OHD levels for the studied pre-pubertal children and: A) HOMA-index (Pearson r=-0.26, P>0.05); B) FBI levels (Pearson r=-0.26, P>0.05).

We stratified the studied children into two groups by WC according to the clinical cut-off for abnormal levels using the Bulgarian reference for children and adolescents into two groups: not abdominally obese < 90-th percentile, abdominally obese ≥ 90-th percentile.[19] Significant differences between non-obese (31.25±7.34kg) and obese group (47.39±11.03kg) were observed for body weight, P<0.001; WC (65.48±5.92cm vs. 82.95±8.41cm, P<0.001) and PTH levels (16.49±11.56 vs 33.07±31.99, P<0.001). Despite all parameters related to glucose and lipid metabolism were in their reference ranges unfavourable but still non-significant changes were observed in the obese group. Most pronounced changes were noted for TC, increase by 23%, and for HDL-cholesterol, decrease by24% and thus the ratio TC/HDL-cholesterol was increased by 57%. Although non-significant, the reduced levels of Ca and 25OHD in the obese group result in significant increase of iPTH: 16.49±11.56 vs. 33.07±31.99ngr/L, P<0.001.

Serum C-reactive protein (CRP) was used as indicator for chronic inflammatory response related to obesity. Despite the non-significant difference between the obese and non-obese group (3.91±3.81 vs. 2.24±2.55mg/L) the increase in CRP levels in the obese group are closer to the upper reference limit.

DISCUSSION

Considerable amount of research reveals that vitamin D deficiency is epidemic worldwide not only among adults but also in children.[20,21]

The best indicator of vitamin D status is serum 25OHD concentration.[22] Most commonly, vitamin D deficiency is defined as 25OHD serum concentrations below 50nmol/L and severe vitamin D deficiency less than 25nmol/L.[23,24] Comparing our results with the accepted cut-off values for vitamin D deficiency, we found a borderline value of 49.65nmol/L for the entire studied population, indicating that Bulgarian pre-pubertal children are at risk of development vitamin D deficiency. Until now, there is no published data on the vitamin D status in Bulgarian pre-pubertal children. According to Spiro and Buttriss[25] the mean year-round levels of 25OHD were 48.0nmol/L for UK pre-pubertal girls aged (4-10 years) and 52.3nmol/L for UK pre-pubertal boys. Our preliminary results show very close values for Bulgarian pre-pubertal girls and boys to those from UK – 45.09nmol/L and 52.80nmol/L, respectively. Higher rate of vitamin D deficiency was established among the pre-pubertal girls when compared to pre-pubertal boys, 65% vs 51.7%. Similar results for vitamin D status were found for Turkish and US children.[4,26] A prevalence of severe vitamin D deficiency among girls (15%) than in boys (3.4%) was found when the participants were categorized by the degree of vitamin D deficiency.

It is well known vitamin D status shows a considerable seasonality, related to variations in the intensity of solar

UV-B radiation with the season.[27] Our data are in agreement with the lower values for 25OHD in winter found by others.[25,28,29] Sioen et al found a significant difference in 25OHD concentration between winter and spring or summer samples.[28] Similarly vitamin D deficiency in more than 50% of school age children and adolescents in winter was reported by Shin et al.[29] More over our results indicated relatively higher values for boys during winter compared to girls (P=0.06), whereas during summer such tendency was not observed.

Additional factor determining the vitamin D variations among children is the body fat deposition. It is considered that vitamin D, being fat-soluble, can be sequestrated by fat tissue.[30] Widely used anthropometric parameter for categorization of thinness, overweight and obesity in children using international cut-offs is the BMI. A disadvantage of this parameter is that it does not distinguish between lean and fat mass.[31] Another simply measured anthropometric parameter is WC that correlates with visceral adiposity in children and increases with the deposition of fat. WC is recognized as a predictive risk indicator for IR, dyslipidaemia and diabetes in children.[32]

In the present study, we found that from the parameters defining whole-body obesity (BMI) and abdominal fat (WC) the latter differs significantly between 25OHD deficient and sufficient groups. Vitamin D deficiency (25OHD<50nmol/L) relates to a larger WC (P=0.055). Studies on Belgian (4-11 years) and Spanish children (9-13 years) concluded that 25OHD correlate significantly with BMI and WC and these parameters influenced the appearance of vitamin D insufficiency (25OHD between 50 and 75nmol/L) in children.[28,33] The lack of statistical significance between 25OHD groups and BMI in our study may be due to the small number of studied children, which is one of its limitations. Examining the relationship between 25OHD and WC, we established a trend for negative correlation (Pearson r =-0.38, P>0.05). Sioen et al found a similar correlation between 25OHD and WC (Pearson r = -0.108, P<0.05) in Belgian children.[28]

There are several mechanisms by which 25OHD can affect glucose homeostasis. As a regulator of calcium levels 25OHD may affect insulin secretion and signalling by calcium-dependent manner.[34,35] Considering the negative impact of systemic inflammation on insulin sensitivity, it is proposed that sufficient 25OHD, due to its immunomodulatory effects, may prevent IR.[36]

Aypak et al reported a significant inverse correlation between serum 25OHD and HOMA-IR in pubertal obese children, but not in pre-pubertal.[26] In our study the correlation analyses showed inverse trend between serum 25OHD and HOMA-index (Pearson r = -0.26), serum 25OHD and FBI levels (Pearson r = -0.26) for the entire studied group. The same non-significant tendency for both parameters was observed when the participants were stratified by gender. Study on Asian-Indian children and adolescents (6-17 years) reveals also inverse relationship trends between 25OHD, glucose, and insulin. The authors did not establish statistically significant correlation between 25OHD and parameters of insulin sensitivity or resistance.[37]

Inflammatory cytokines produced in visceral fat cause elevation of serum C-reactive protein (CRP), which is reported to be positively correlated with some metabolic alterations.[38] CRP significantly increases in obese children compared to normal-weight, and positively correlate with BMI.[39] However, the increase of CRP is lower than during acute episodes of inflammation or infection. Significant increase in CRP is found mainly in cases of severe (morbid) obesity. In our study only 2 children have BMI above 30 kg/m^2 which could explain the lack of significant increase of CRP in obese children compared to non-obese.

Strengths and limitations

The present pilot study is the first attempt to evaluate the vitamin D status in Bulgarian pre-pubertal children and to examine its relationships with parameters of whole body obesity and IR. To assess vitamin D status we used HPLC assay – the most robust method for measuring the serum concentration of 25OHD.[40] One of the limitations of the study is the small number of enrolled patients. Another is that the degree of adiposity was assessed by BMI and WC which may not truly reflect body fat status. The observed gender differences in vitamin D status may be due to the different physical activity and dietary intake between boys and girls which is not assessed in our study.

CONCLUSIONS

This pilot study shows that half of the studied Bulgarian pre-pubertal children were vitamin D deficient, more prevalent in the winter. The preliminary negative trends found between serum 25OHD levels and WC, as a parameter of abdominal fat, HOMA-index for IR, and serum insulin concentrations may provide grounds for further larger studies to examine these associations in more depth.

ACKNOWLEDGEMENTS

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