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Research Paper

Low Vitamin D Status of Northern Italian Children in Pediatric Primary Care Setting: What to Do?

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ABSTRACT

Aims: To analyze vitamin D status in a group of children living in Northeastern Italy cared by a "family pediatrician".
Study design: Cross-sectional study.
Place and Duration of Study: Pediatric primary care in a rural area near Padua (Italy, 45°N latitude), between November 2010 and September 2012.
Methodology: The study was conducted with 113 children (41 girls and 72 boys), aged between 1 and 15 years old. The serum level of 25-hydroxyvitamin D [25(OH)D] was measured using a chemiluminescence immunoassay methodology. Serum 25(OH)D test was included in a panel of laboratory tests ordered for different reasons. A correlation was researched between 25(OH)D level and the following variables: class of age, gender, ethnicity, skin colour, period of blood withdrawal, BMI category, results in other laboratory tests and presence of comorbidity.
Results: Only 26,5% of children had a normal level of 25(OH)D ($\geq$ 30 ng/ml); in 66,4% of all patients 25(OH)D level was 10-29 ng/ml while 7,1% of children had 25(OH)D < 10 ng/ml. About 40% of all children had 25(OH)D < 20 ng/ml. Non-Italian ethnicity, non-white skin and blood withdrawal in January-March and April-June were significantly associated with hypovitaminosis D [25(OH)D < 30 ng/ml] at univariate level. Both non-Italian ethnicity (P = 0.029) and period of blood withdrawal (P = 0.0062) were also significant at multivariate analysis. The combination of chronic disease *or* non-white skin could identify only 50% of children with 25(OH)D < 10 ng/ml and 29% of children with 25(OH) < 20 ng/ml.
Conclusion: We noted a high incidence of hypovitaminosis D in asymptomatic children without risk factors. In our region cholecalciferol supplementation should be implemented for all children between October and April. Appropriate dose for children of Northern Italy is debated.

Keywords: Vitamin D, cholecalciferol, 25-hydroxycholecalciferol, hypovitaminosis D, vitamin D deficiency, vitamin D insufficiency, seasonal variation.

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1. INTRODUCTION

Hypovitaminosis D is a common problem worldwide in children and adolescents [**1-4**], as well in adults [**5-6**], that places affected population at high risk for chronic diseases [**7**]. Although severe vitamin D deficiency is rare, there is accumulating evidence of the frequent occurrence of subclinical vitamin D deficiency or insufficiency in otherwise healthy people [**8-14**]. To our knowledge there are only a few studies on children living in Northeastern Italy [**15-17**]: they have been conducted retrospectively [**15**] or examining patients afferent to a Pediatric Department [**16**] or asthmatic [**17**]. On this basis an analysis of vitamin D status was prospectively conducted measuring serum 25-hydroxyvitamin D [25(OH)D] in a group of children living near Padua in a primary care setting.

2. MATERIALS AND METHODS

2.1 Patients and methods

A cross-sectional study was conducted in 113 children living in a rural area near Padua (Italy, 45°N latitude) and cared in office by a single *family paediatrician.* These patients represented 12% of about 900 children registered in his list. The 25(OH)D test was included in a panel of laboratory tests (lab tests) ordered for different reasons (suspected anemia, poor growth, fatigue, etc.) between November 2010 and September 2012. The children weren't taking vitamin D before blood collection and there was no official policy of Italian National Health Service on vitamin D prophylaxis in children > 12 months. Children aged < 12 months were excluded. In Italy they usually receive 400 IU cholecalciferol per day.
The serum level of 25(OH)D was measured using a chemiluminescence immunoassay methodology. The laboratory normal minimum was 30 ng/ml; values of 29 ng/ml or less were considered as hypovitaminosis D: laboratory defined insufficiency as 10-29 ng/ml and deficiency as < 10 ng/ml.
Levels above 100 ng/ml were considered as hypervitaminosis D. Toxicity (including hypercalcemia and hyperphosphatemia) generally occurs for serum levels of 25(OH)D greater than 150 ng/ml [**18**].
Data collected on patients included age, gender, self-declared ethnicity, skin colour, body mass index (BMI), results of other lab tests and presence of comorbidity. Diet was not analyzed but none of the children was vegetarian. Children were divided into the following age groups: children between 1-5 years old (preschoolers), 6-10 years old (schoolers) and 11-15 years old (adolescents). BMI was calculated and children were divided into 4 categories considering their BMI-for-age-and-gender percentile: underweight (BMI less than 5^{th} percentile) [**19**], healthy weight (BMI from 5^{th} percentile to less than 85^{th} percentile), overweight (BMI from 85^{th} to less than 95^{th} percentile) and obese (95^{th} percentile or greater) [**20**].

2.2 Statystical Analysis

All data collected were analyzed and described using descriptive statistics. Absolute and percent frequencies were used for qualitative variables; mean, standard deviation (SD), range and median were used to summarize quantitative variables. Principal data were also stratified by level of 25(OH)D (< 10 ng/ml, 10-29 ng/ml, and $\geq$ 30 ng/ml). Chi-squared (univariated analysis) test was used to determine the presence of a relationship between 25(OH)D stratification groups (deficiency, insufficiency, and normal level) and the following variables: month of blood withdrawal, class of age, gender, ethnicity, skin colour, BMI category, results of other lab tests (e.g. haemoglobin, serum iron, serum cholesterol) and presence of comorbidity. The linear correlation between quantitative variables (BMI and

month of blood withdrawal) and 25(OH)D level was also tested by Pearson's correlation coefficient (ρ). Multinomial logistic regression was used to test factors influencing the probability of hypovitaminosis D [25(OH)D < 30 ng/ml]. The analysis of variance (ANOVA) was used to verify the different level of 25(OH)D in different periods. Tests were considered significant at $P < 0.05$ level. SAS® software (SAS Institute Inc., Cary, North Carolina, USA) was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Demographic characteristics

Between November 2010 and September 2012, 25(OH)D level was measured in 113 children. Their demographic characteristics are represented in Table 1.

Table 1. Demographic characteristics of 113 children

Characteristics	N.
Females / Males	41 / 72
1-5 years	53
6-10 years	37
11-15 years	23
Italians / Non Italians	91/22
White / Non white skinned	101/12

3.1.2 Prevalence of Hypovitaminosis D

Values of serum 25(OH)D varied from 4 to 70 ng/ml; the mean level was 24.2 ng/ml (SD 11,6 ng/ml; median 22.0 ng/ml; interquartile range 17-30 ng/ml). Only 30 children (26.5 %) had a normal level; in 75 children (66.4%) serum 25(OH)D was 10-29 ng/ml, and 8 children (7.1%) had 25(OH)D < 10 ng/ml (Table 2).

Table 2. Distribution of children by level of 25(OH)D

25(OH)D level	N.	%
< 10 ng/ml	**8**	**7.1**
10-29 ng/ml	**75**	**66.4**
10-19	37	32.8
20-29	38	33.6
≥ 30 ng/ml	**30**	**26.5**
All	**113**	**100**

3.1.3 Gender

There were 41 females (36.3% of all patients) and 72 males (63.7%). No statistically significant difference was found between males and females regarding the levels of 25(OH)D: 3 females (7.3% of females) and 5 males (6.9% of them) had 25(OH)D < 10 ng/ml; 28 females (68.3% of them) and 47 males (65.3% of males) had 25(OH)D 10-29 ng/ml. Only 10 females (24.4% of females) and 20 males (27.8% of males) had a normal level of 25(OH)D (≥ 30 ng/ml).

3.1.4 Age group

53 children (46.9% of all patients) were 1-5 years old (preschoolers), 37 (32.7%) were 6-10 years old (schoolers) and 23 (20.4%) were 11 years old or older (adolescents).
Children aged 5 years or less had more frequently a level of 25(OH)D < 10 ng/ml comparing with other groups, even if there was not statistical significance (Table 3).

Table 3. Relationship between age group and 25(OH)D level

	All		25(OH)D level					
			< 10 ng/ml		10-29 ng/ml		≥ 30 ng/ml	
	N	% col	N	% row	N	% row	N	% row
1 - 5 y	53	46.9	6	11.3	31	58.5	16	30.2
6 - 10 y	37	32.7	1	2.7	28	75.7	8	21.6
11 - 15 y	23	20.4	1	4.3	16	69.6	6	26.1
All	113	100.0	8	7.1	75	66.4	30	26.5

Chi-Square: P = 0.3785

3.1.5 Body Mass Index

Most of the children (71.7%) had normal BMI-for-age, with similar distribution for girls (65.9%) and boys (75%). A total of 15% of the children were overweight (26.8% of females and 8.3% of males) while another 4.4% were obese (2.4% of females and 5.6% of males).
A relatively small percentage (8.8%) of the children was underweight for age (4.9% and 11.1% of the 72 boys and 41 girls, respectively).
The distribution of BMI according to different age groups was the following: among preschoolers 15.1% were underweight, 75.5% had normal weight, 5.7% were overweight and 3.8% were obese; among children 6 years old or more, 3.3% were underweight, 68.3% had normal weight, 23.3% were overweight and 5% were obese.

Rates of hypovitaminosis D [25(OH)D < 30 ng/ml] were higher in children with low or normal weight (90% and 74.1% respectively) than in overweight and obese children (64.7% and 60% respectively), but this trend did not reach statistical significance (*P* = 0.4661).

3.1.6 Ethnicity

The population of the study was composed by children of different ethnicity: 91 of them were Italian (80.5%), 10 were African (8.8%), 9 were European (7.9%): Albanian (3 children), Moldovan and Romanian (2 children each), Czech and Spanish (1 child each); 2 were American (Central and South), and one child was Chinese. One of them was an adopted boy.

Non-Italian children had a higher risk for 25(OH)D < 10 ng/ml if compared with Italian children: 22.7% versus 3.3% (Table 4).

Table 4. Relationship between ethnicity and 25(OH)D level

	All		25(OH)D level					
			< 10 ng/ml		10-29 ng/ml		≥ 30 ng/ml	
	N	% col	N	% row	N	% row	N	% row
Italian	91	80.5	3	3.3	62	68.1	26	28.6
Non Italian	22	19.5	5	22.7*	13	59.1	4	18.2
All	113	100.0	8	7.1	75	66.4	30	26.5

Chi square: P = 0.0056.

3.1.7 Skin colour

The distribution of skin colour according to vitamin D status showed that there was a statistically significant relation between non-white skin and 25(OH)D < 10 ng/ml (*P* = 0.0361) (Fig. 1).

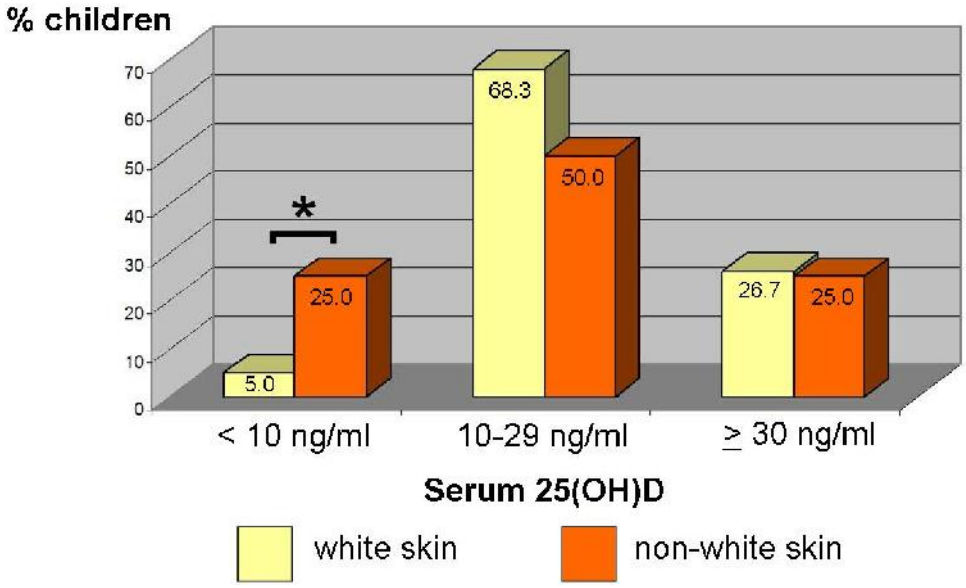

Fig. 1. Vitamin D status according to skin colour

**Non-white children had a significantly higher rate of deficiency than white children (25.0% versus 5.0%; P = 0.0361).*

3.1.8 Period of blood withdrawl

There was a seasonal effect on 25(OH)D levels that is represented in Figure 2.

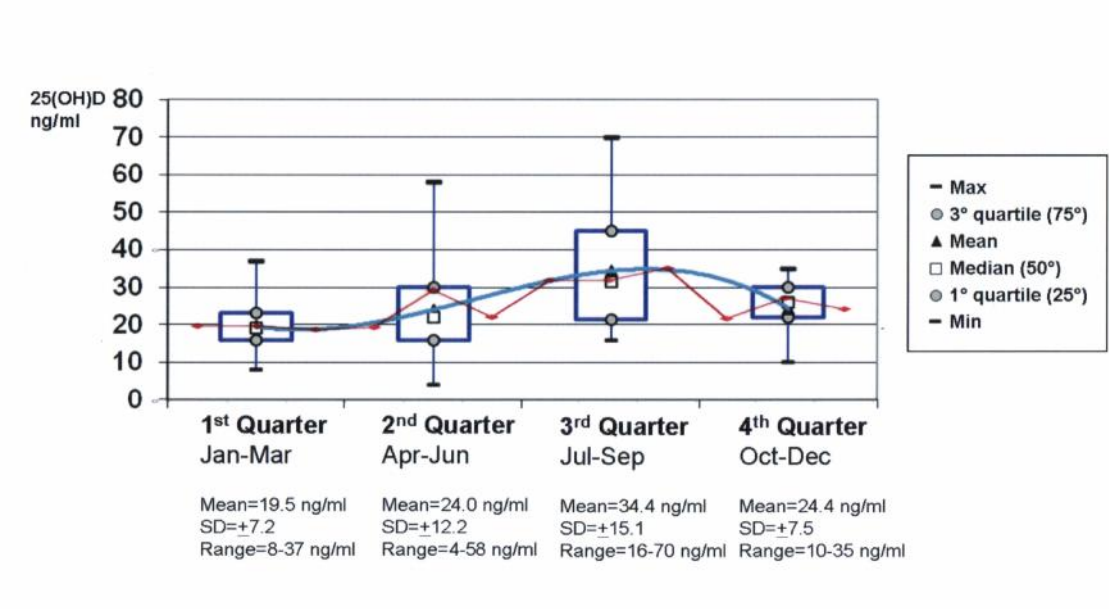

Fig. 2. 25(OH)D level according to period of blood withdrawl.
Bend line: Quarterly trend. Broken line: monthly level.
Analysis of variance: P = 0.0003

Children with 25(OH)D < 10 ng/ml were identified only in the first and in the second quarter of the year. When serum samples were withdrawn between January and March only 11.8% (4/34) of children showed a normal 25(OH)D level, while in 76.5% (26/34) it was 10-29 ng/ml and in 11.8% (4/34) it was < 10 ng/ml. In the second period (April-June), a normal level of 25(OH)D was found in 26.7% of children (12/45); 25(OH)D was 10-29 ng/ml in 64.4% of them (29/45) and it was < 10 ng/ml in 8.9% (4/45). Only samples withdrawn in the third quarter (July-September) showed mean concentrations of serum 25(OH)D $\geq$ 30 ng/ml. The higher rate of children with normal 25(OH)D, 50.0% (8/16), was found in the third period (July-September); the remaining 50% had 25(OH)D 10-29 ng/ml. In the fourth quarter (October-December) 33.3% of children had normal 25(OH)D (6/18) and the remaining 66.7% had 25(OH)D 10-29 ng/ml (12/18). Analysis of variance showed a statistically significant difference between periods of blood withdrawal (P = 0.0003).

3.1.9 Serum 25(OH)D and other laboratory tests

Serum 25(OH)D test was included in a larger panel of lab tests together with haemoglobin, white blood cells, serum iron, serum cholesterol and others. Parathyroid hormone (PTH), alkaline phosphatase, calcium and phosphorus were done only in a few children because this was not a structured study and its purpose wasn't to analyze biochemical consequences of hypovitaminosis D.
Eighty-two percent of the children with no alteration in other parameters had 25(OH)D under 30 ng/ml compared with 66.7% of children with at least one value altered, among those considered, this difference being not statistically significant (P = 0.0668).

3.1.10 Comorbidity

In this study we considered the relationship between 25(OH)D stratification groups and the presence of comorbidity (Table 5).

Table 5. Distribution of comorbidity and vitamin D status

	25(OH)D < 10 ng/ml		25(OH)D 10-29 ng/ml		25(OH)D $\geq$ 30 ng/ml		All	
	N	% row	N	% row	N	% row	N	% row
No comorbidity	5	10.2	30	61.2	14	28.6	49	43.4
Comorbidity	3	4.7	45	70.3	16	25.0	64	56.6
All	8	7.1	75	66.4	30	26.5	113	100.0

Chi-Square: P = 0.4335

Only one out of our eight children with 25(OH)D < 10 ng/ml had a chronic illness (he was a coeliac and asthmatic boy). Among 45 children with 25(OH)D < 20 ng/ml there were 7 patients affected by a chronic disease (coeliac disease 3, epilepsy taking specific drugs 2, congenital hypothiroidism 1). The combination of chronic disease *or* non-white skin could identify 4/8 children (50%) with 25(OH)D < 10 ng/ml and 13/45 children (29%) with 25(OH)D < 20 ng/ml. The same combination identified 7/12 children (58%) with 25(OH)D 10 ng/ml or less that is another definition of deficiency [**10**].

The most common comorbidity was atopic disease, that affected 22 children; the other comorbidities were neuropsychiatric disorders (6 children), anemia (5 children), metabolic disorders (5 children), skeletal disorders (4 children), chromosomal syndromes (3 children), hypersensitivity contact dermatitis (2 children), tumors (2 children), precocious puberty (2 children), other conditions (10 children).

3.1.11 Multivariate analysis

Ethnicity and period (quarter of the year) of blood withdrawal, significant at univariate level, were also tested by multivariate analysis to verify the association with hypovitaminosis D. Both non-Italian ethnicity (P = 0.029) and period of blood withdrawal (P = 0.0062) were significantly associated to lower level of 25(OH)D.

3.2 Discussion

We studied a group of 113 children living in a rural area of Northeastern Italy (45°N latitude). All patients attended a single pediatrician office and had blood withdrawn for measurement of serum 25(OH)D, that is the best indicator of overall vitamin D status, because it reflects total vitamin D from dietary intake and sunlight exposure, as well as the conversion of vitamin D from adipose stores in the liver [**21-24**]. Prevalence of hypovitaminosis D in our region is similar to others of "vitamin D winter area" (latitudes above 37 degrees North and South) [**8, 9, 25**].

3.2.1 Vitamin D status in children

As reported in the literature, patients with vitamin D deficiency are much less frequent than those with insufficiency. Our prevalence of 7.1% deficient children is similar to that reported in NHANES Study 2001-2004 that found a rate of 9% [**26**]. It is also remarkable that two among three of our children had an insufficient vitamin D status, that is about the same as that shown in US children in the study mentioned above [**26**]. Moreover, about 40% of our patients had serum 25(OH)D < 20 ng/ml, that is the cut-off recently suggested to diagnose vitamin D deficiency [**10, 27-31**] A similar prevalence of serum 25(OH)D < 20 ng/mL – 42% – was reported in a sample of healthy US adolescents [**11**].
The importance of these data is capital because 25(OH)D is a fundamental prehormone [**32**]; in fact Vitamin D receptor is expressed in nearly all tissues and affects the transcription of over 900 genes in human cells [**33**], being critically important for the development, growth, and maintenance of a healthy body in humans.

3.2.2 Risk factors for hypovitaminosis D

In our group of children factors consistently associated with low vitamin D status included blood withdrawal in January-June, non-white skin colour and non-Italian ethnicity.
Mean level of 25(OH)D was < 30 ng/ml in October-December and < 20 ng/ml in January-April. In other studies a high risk of hypovitaminosis D was found only in winter season [**8, 34**], while in a population based study blood levels taken December-May in Great Britain had an Odd Ratio of 6.5 for vitamin D insufficiency in UK children [**9**]. The angle of sunlight during winter above 37 degrees latitude North is insufficient to stimulate the skin to make vitamin D, and this effect drifts in the early spring.
In our group non-Italian children had a significantly higher rate of vitamin D deficiency and this was correlated with the presence of non-white skin, as reported in the literature [**8, 9**]. In fact skin pigmentation blocks the absorption of ultraviolet sunlight and decreases vitamin D synthesis from photo-conversion of cutaneous 7-dehydrocholesterol. Nevertheless, it cannot

be ruled out that the socioeconomic status rather than ethnicity or skin colour was responsible for this difference.
Another risk factor of hypovitaminosis D is lack of sun exposure; we can find a typical example of this condition in adopted children due to prolonged institutionalization in pre-adoptive period [**35**].
The relationship between hypovitaminosis D and BMI is controversal; for example it may be associated with overweight [**8-10**] or underweight [**36**], and the latter is also our experience: in fact 9 out of 10 underweight children had 25(OH)D < 30 ng/ml.
Some authors had found higher rates of hypovitaminosis D in children with greater fat mass and higher BMI [**8, 34, 37, 38**]. In a study the prevalence of vitamin D deficiency increased significantly with BMI: it was 21% in healthy-weight, 29% in overweight, 34% in obese, and 49% in severely obese children [**39**]. Because of vitamin D deposition in fatty tissue, it was suggested that the larger storage capacity in obese people may prevent 25(OH)D from circulating in the bloodstream [**40**]. Mechanism is probably more complex and includes other factors besides the decreased bioavailability, because hypovitaminosis D really precedes overweight [**41**]. Vitamin D status in underweight children with low serum 25(OH)D is probably different because these patients have both the circulating and the stored vitamin D levels poor. This depends in part on the limited quantity of vitamin D precursors in the skin due to the slim layer of fatty tissue. The fact that not always underweight children have hypovitaminosis D may be due in part to the different use of vitaminic supplements: in fact children classified as being underweight or at risk of underweight had a higher prevalence of use of vitamin D-containing supplements than did children classified as at risk of overweight or overweight [**42**].
Some authors reported that adolescence is significantly associated with hypovitaminosis D [**8, 9**], while in our experience preschool children had more frequently a level of 25(OH)D < 10 ng/ml comparing with older children, even if there was not statistical significance. This trend may depend in part on the higher rate of underweight children in this class of age and in part on the fact that little children rarely go outdoors without parents surveillance.
Other factors (e.g., estimated sunlight exposure, sunscreen use, diet, etc.) were not analyzed because the purpose of our "field-study" was to examine vitamin D status of children in a primary care setting and not to determine in detail the cause of the observed levels.

3.2.3 To screen or not

Holick suggested that only a few categories of patients should be screened for vitamin D deficiency/insufficiency and monitored for their 25(OH)D concentration while being treated with vitamin D, that are patients with malabsorption (inflammatory bowel disease, cystic fibrosis), and liver and kidney diseases; patients taking antiseizure medications, glucocorticoids, rifampicin, isoniazid or AIDS medications, patients with primary hyperparathyroidism or chronic granulomatous disorders [**27**]. On this basis, taking into account also coeliac disease, we would have 7 out of 8 children with vitamin D deficiency and 38/45 with 25(OH)D < 20 ng/ml undiagnosed. Considering the combination of chronic disease *or* non-white skin we would have missed 50% of children with 25(OH)D < 10 ng/ml and 71% of children with 25(OH)D < 20 ng/ml. Also lab tests other than 25(OH)D weren't predictive of hypovitaminosis D: in fact a level of 25(OH)D < 30 ng/ml was relatively more frequent in children with no alteration in other lab tests. So neither clinical/demographic factors nor lab tests other than 25(OH)D could identify all children with hypovitaminosis D. Holick suggested that it could be more cost-effective to implement a vitamin D supplementation program for all children and adults than to measure everybody's serum 25(OH)D [**27**] but the question now is how much vitamin D should be given.

3.2.4 How much vitamin D must be given and when

The dose to give as supplement depends both on baseline and target serum level of 25(OH)D [**43**]. There is no general agreement on which level is needed for an optimal health condition, even if some medical societies had stated 20 ng/ml as the minimum desirable serum 25(OH)D level during evolutive age [**28-31**].

A Recommended Dietary Allowance (RDA) of 600 IU per day ensures a 25(OH)D serum level of 20 ng/ml to 97-98% of population aged 1-18 years [**30**]. Moreover some authors suggested higher levels: 30 ng/ml [**44, 45**], 40 ng/ml [**31, 46**] or even 60 ng/ml to obtain the maximum health benefits [**47, 31**]. Some recommendations are derived from adults data, because little is still known about requirements of vitamin D in children. Some studies showed that 25(OH)D levels < 30 ng/ml are associated with bone demineralization [**48**] and an increase in PTH [**49**]. The target for optimal health depends on personal situations or life periods; the question is far more complex because the relationship between 25(OH)D level and effect on health can be nonlinear: emerging evidence suggests a U-shaped curve for several outcomes related to vitamin D in adults [**30**] and also in pediatric age [**50**]. In fact serum 25(OH)D levels above 50 ng/ml had potential risks for some outcomes [**30**].

Assuming that most of our children had less or much less than the minimum desirable [**44**] and considering that for every 100 IU of vitamin D taken in, there is an increase of rough 1 ng per milliliter in the serum level of 25(OH)D [**51, 52**], recommended doses of 600 IU per day [**30, 53**] probably offer no advantage when treating children with 25(OH)D < 10 ng/ml and even < 20 ng/ml. NHANES 2001-2006 data has demonstrated that 10% of children taking vitamin D supplements at doses 100-400 IU per day had a 25(OH)D level < 20 ng/ml and over 50% of children had a level < 30 ng/ml [**1**]. In an intervention study, 38% of peripubertal Finnish girls with vitamin D_2 supplementation approximately 800 IU daily from October to January had moderate hypovitaminosis D [25(OH)D 8-15 ng/ml] after treatment [**54**].

There is accumulating evidence to suggest that vitamin D intake should be in the range of at least 800-1,000 IU per day [**50, 55**]. The evidence-based recommendations by the Endocrine Society's Clinical Practice Guidelines stated 400-1,000 IU for children, 1,500-2,000 IU for adults to maintain 25(OH)D concentrations of 40-60 ng/ml for preventing and treating vitamin D deficiency [**49, 56**]. Holick recommends 1,000-1,500 IU/day for children 1-10 years of age, and 1,500-2,000 IU/day for teenagers [**22**].

While the Institute of Medicine Committee in 2011 recommended 600 IU vit D per day from 1 to 70 years of age [**30**] and also Italian LARN revised 2012 recommended 600 IU per day as for children aged 1-17 years as for adults aged 18-74 years [**57**], recent guidelines of the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS) [**58**] highlighted that healthy adults with no effective sun exposure should be supplemented with 1,200 IU vitamin D per day. In our opinion children living in Northeastern Italy need about 1,500 IU cholecalciferol per day from October to April, that is about a half of tolerable (safe) upper limit for vitamin D [**30**]; higher doses are probably necessary in non-white skinned children.

Further studies are necessary in order to confirm vitamin D needs of Northern Italian children.

4. CONCLUSION

We noted a high incidence of hypovitaminosis D in asymptomatic children living in Northeastern Italy without risk factors. In our region cholecalciferol supplementation should be implemented for all children between October and April.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTION

This work was carried out in collaboration between all authors. Author SM conceptualized and designed the study, did acquisition and analysis of the data, drafted the initial manuscript, and approved the final manuscript as submitted. Author CB participated in the collection of the data, in the drafting of manuscript and final approval of the manuscript. Author DT participated in the interpretation of the data, critical evaluation of the manuscript and final approval of the manuscript. All authors read and approved the final manuscript.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not Applicable

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