CLINICAL STUDY

High prevalence of genu varum/valgum in European children with low vitamin D status and insufficient dairy products/calcium intakes

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Abstract

Objective: The prevalence of lower limb deformities physiologically decreases after 5 years of age. It remains high in some tropical and subtropical regions where it has been associated with severe vitamin D deficiency, low calcium/milk intakes, malnutrition, and/or fluoride overexposure. Very little data is available in apparently healthy Caucasian children and adolescents.

Design: We evaluated the prevalence of genu varum/valgum and other clinical symptoms, and assessed vitamin D status and markers of calcium metabolism in 226 apparently healthy European full-time boarders (7–16 years) seen during winter–spring and fed a cereal-based diet with little access to meat, milk, and dairy products. A cohort of 71 white children and adolescents hospitalized for acute illness served as age-matched controls.

Results: Association studies showed a high prevalence of lower limb deformities (36%) and higher alkaline phosphate activities in the 21% of children and adolescent full-time boarders with serum 25-(OH)D levels ≤ 30 nmol/l, and low serum calcium in the 74% of boarders with 25-(OH)D levels ≤ 50 nmol/l, compared with boarders with higher vitamin D status. No such anomalies were found in the control cohort despite lower serum 25-(OH)D levels.

Conclusions: Low 25-(OH)D levels, at least during winter–spring, combined with additional risk factors such as very low calcium/milk intakes and possibly digestive disorders, are associated with an increased risk of genu varum/valgum in European children and adolescents. Thus, dietary fortification, or supplementation with vitamin D, may be recommended, at least during the winter, to European children and adolescents with either none or insufficient calcium/dairy product intakes.

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Introduction

Serum 25-(OH)D levels are considered the best marker of vitamin D status and have been commonly used to assess the prevalence of vitamin D deficiency/insufficiency in healthy children and adolescents (1–9). But a clear understanding of the clinical significance of given concentrations of circulating 25-(OH)D levels is still lacking (10–13), and the vitamin D status needed for an optimal bone health and calcium homeostasis during growth is still in discussion, with proposed 25-(OH)D cutoff values ranging from 25–30 to 70–90 nmol/l (2, 6–9, 14–16).

To define an optimal vitamin D status necessitates the systematic use of external international controls to test the validity of the 25-(OH)D assay and permit comparisons of the results obtained in the different

laboratories (13, 17). It may also depend on the criteria selected to evaluate bone health, either biological (levels of serum calcium, serum PTH, and/or of markers of bone metabolism), radiological (bone mineral content or density at appendicular or trabecular sites), or, less frequently, clinical (lower limb deformities for example). So far, the lower limb deformities attributed to vitamin D and/or calcium deficiency have mostly been observed in children and adolescents living in Africa, Asia, or the Middle East (11, 18–24), and the sporadic cases reported in Europe, America, and Australia habitually concern recently immigrated children or offspring of immigrant parents from Asia, Africa or the Middle East (11, 25, 26). Its prevalence is considered to be low in children and adolescents of Caucasian origin (27, 28), but studies are scarce and possible interplays with calcium intakes have not been considered, despite the

emerging evidence that calcium deficiency may lower the threshold under which vitamin D status becomes insufficient to ensure an optimal mineralization of the growing skeleton (2, 11). In the present work, we evaluated lower limb deformities and biochemical markers of calcium metabolism in a homogeneous cohort of 226 European children and adolescents receiving a cereal-based diet with low milk/high fiber intakes. We also measured serum 25-(OH)D levels, using the International External Quality Assessment Scheme for Vitamin D metabolite (DEQAS), in order to estimate optimal levels of 25-(OH)D for bone health in Caucasian children with very low calcium/dairy product intakes.

Subjects and methods

Subjects and recruitment

The study included all ($n=226$) apparently healthy children and adolescents (97 girls and 129 boys, aged 7–16 years) seen in the course of a regular medical and biological survey during the winter–spring months (January to April) while staying as full-time boarders in a Moldovan boarding school (Orhei, latitude, 47°N). They stayed at this boarding school during the academic year, but lived in a rural family environment in the summer. Only children with known chronic disease and/or body measures below or above 2 s.d.s were excluded from the study. Seventy-one age-matched controls (34 girls and 37 boys) had an identical clinical and biochemical survey while hospitalized during the same period for acute respiratory diseases in the Municipal Clinical Children's Hospital n°1 of Chisinau (Moldova, 47°21'N). They lived in a family of urban environment and had no known renal, endocrine, neurologic, or other chronic pathology.

All blood samples were collected during a systematic, diagnostic, or hematological survey, and approval of a local ethical committee, as well as oral consent of the children, their parents, or teachers was obtained.

Dietary intake

Full-time boarders were given one unique menu at each meal, including weekends. They had a cereal-based diet with little access to meat, milk, and dairy products. The CIQUAL table of food nutritional composition (<http://www.afssa.fr/TableCIQUAL>) was used to evaluate dietary intakes from 1 week menus given to all boarders at the time of the survey. Average daily intakes were the following: – total protein, 75 g (2.5 ± 0.4 g/kg b.w per day for children and 1.8 ± 0.3 g/kg b.w per day for adolescents), including animal proteins, 24 g; energy, 2842 kcal; phosphates, 921 mg; magnesium, 255 mg. Calcium daily intakes were low (493 mg including 69 mg as milk and dairy products),

but vitamin D intakes were high (1.23 g). Dietary information was not available for the hospitalized controls seen in Chisinau.

Clinical examinations and anthropometric measurements

One of the authors (AV) examined all 297 children and adolescents. Bone densitometry was not available, and skeletal X-rays were not performed in healthy boarders for ethical reasons, but intercondylar (IC) and intermalleolar (IM) distances were measured in them standing with either knees or ankles just touching: genu varum or valgum was defined as IC or IM > 5 cm. Term, birth height, and weight were drawn from individual files available for each child. Height and body mass index (BMI) were evaluated both as absolute value and as z-scores, in comparison to WHO reference growth curves for sex- and age-matched children (29). Several other clinical features were noted, including bone pain and scoliosis (Table 1), and the presence of gastrointestinal

Table 1 Clinical characteristics of the two Moldovan cohorts. Values are expressed as mean $\pm$ s.d. or as percentage of subjects with clinical symptoms or intestinal parasitosis in the two study groups. Comparisons were made using the Student's unpaired *t*-test (mean values) or the χ^2 -test (incidence).

Population	Rural boarding school	Urban controls	<i>P</i> values
<i>n</i>	226	71	
Age (years)	11.4 $\pm$ 1.8	11.1 $\pm$ 2.4	
Prepubertal (%)	52%	46%	
Sex (% males)	57%	52%	
Birth parameters			
Term (weeks)	39.4 $\pm$ 0.8	39.2 $\pm$ 1.6	
Birth weight (kg)	3.11 $\pm$ 0.42	3.08 $\pm$ 0.56	
Birth length (cm)	50.5 $\pm$ 2.3	50.4 $\pm$ 2.1	
Anthropometry			
BMI (WHO z-score)	–0.205 $\pm$ 1.041	–0.471 $\pm$ 1.141	
Children (<i>n</i>)	–0.325 $\pm$ 1.107 (118)	–0.261 $\pm$ 1.228 (33)	
Adolescents (<i>n</i>)	–0.440 $\pm$ 0.898 (108)	–0.682 $\pm$ 1.023 (38)	
Height (WHO z-score)	–0.721 $\pm$ 1.054	–0.024 $\pm$ 1.051	< 0.0001
Children	–0.088 $\pm$ 0.964	–0.155 $\pm$ 1.101	
Adolescents	–1.009 $\pm$ 1.126	0.106 $\pm$ 1.000	< 0.0001
Clinical symptoms (%)			
Bowed legs	15	7	
Scoliosis	11	8	
Bone pain	8	3	
Hypocalcemic seizure	1	4	
Poor dental health	83	80	
Dental caries	10	11	
Gastrointestinal	66	75	
Intestinal parasitosis (%)	49	63	
<i>Enterobius vermicularis</i>	40	20	0.0017
<i>Ascaris lumbricoides</i>	4	14	0.0104
<i>Trichuris trichiura</i>	2	14	< 0.0001
<i>Giardia lamblia</i>	–	15	
Polyinfection	3	–	

parasites was investigated using standard coprological methods for *Enterobius vermicularis*, *Giardia lamblia*, *Ascaris lumbricoides*, and *Trichuris trichiura*.

Biochemical analyses

Laboratory variables were assayed in the Chisinau hospital and included serum calcium, phosphate, magnesium, protein, creatinine, hemoglobin, glucose, alkaline phosphatase, and ALT/AST transaminase activities. Serum 25-(OH) vitamin D levels were assayed in serum samples stocked at -20°C at the Saint Vincent de Paul Hospital (Paris) using in-house chromatographic competitive protein binding assays with the DEQAS continuous external quality assessment of the 25-(OH) vitamin D assays (30). Samples were measured together with 20 DEQAS controls (range: 9–79 nmol/l). Values were -0.11 ± 0.72 s.d. units (mean $\pm$ s.d.) from all the laboratory means for each control. PTH and bone markers were not measured because assays were not available locally and because it could not be established that serum samples were kept at optimum conditions during storage and transport to France.

Statistical analysis

Normally distributed variables are reported as mean $\pm$ s.d. Independent unpaired *t*-test was used to compare pairwise normally distributed continuous variables. Significant differences between groups of <30 subjects were verified by using the non-parametric Mann–Whitney *U* test. Comparisons of prevalence between groups were tested by the χ^2 -test. All analyses were performed using the Statview 5.0 software (Abacus Concepts Inc., Berkeley, CA, USA). A $P < 0.05$ was considered statistically significant.

Results

Clinical and biochemical characteristics

The 226 full-time boarders and 71 hospitalized age-matched controls had comparable anthropometry at birth and before puberty (Table 1). But male and female adolescent boarders had shorter height, compared with hospitalized controls and to WHO reference growth curves. The two Moldovan cohorts had poor dental health and a high prevalence of gastrointestinal symptoms, mainly multiple, but also isolated nausea (17%) and isolated abdominal pain (20%). Parasitic infections were frequent, with significant differences in the parasites identified (Table 1). Both populations showed a low prevalence of scoliosis and bone pain (below 10%), but genu varum/genu valgum was frequently observed in the boarding school (15%), even though the difference was not significant with the incidence observed in the control cohort (7%).

Compared with age-matched controls, boarders had lower mean serum levels of calcium, magnesium, and hemoglobin, and higher mean serum levels of phosphates (Table 2). In addition, boarders had lower serum creatinine levels before puberty, and higher alkaline phosphatase activity during puberty, compared with age-matched controls (Table 2). A search for hepatitis B in the boarding school had been negative one year before the present study, but a transitory liver impact of the mebendazole treatment, received by the boarders for their parasitosis 2 weeks before the blood test, cannot be excluded, thus complicating the interpretation of circulating alkaline phosphatase activities in this cohort. Yet, no correlation was observed between serum alkaline phosphatase and AST, or ALT, transaminase activities in boarders or controls before or during puberty.

25-(OH)D status

Children and adolescents seen in the rural boarding school between January and April had a mean $\pm$ s.d. serum 25-(OH)D levels of 44 ± 16 nmol/l, with respective prevalence of 25-(OH)D values ≤ 30 , ≤ 50 , and ≤ 75 nmol/l of 21, 74, and 97%. This vitamin D status was higher than that measured in control children and adolescents living in the Chisinau city, either in apartment (35.6 ± 1.8 nmol/l) or individual houses (37.0 ± 2.3 nmol/l). It was not influenced by pubertal maturation but was influenced by sex: female boarders had lower mean values than males (40.2 ± 14.8 vs 46.5 ± 16.0 nmol/l, $P = 0.0031$) and a higher prevalence of 25-(OH)D levels ≤ 30 nmol/l (30 vs 15%, $P = 0.0093$).

Table 2 Biochemical profile (mean $\pm$ s.d.).

Population	Boarding school	Urban controls	<i>P</i> values
<i>n</i>	226	71	
Children (<i>n</i>)	118	33	
Adolescents (<i>n</i>)	108	38	
Proteins (g/l)	69 ± 11	69 ± 8	
Hb (g/l)	11.2 ± 1.1	11.9 ± 0.4	<0.0001
Glucose (mmol/l)	4.2 ± 0.8	4.3 ± 0.7	
Children creatinine ($\mu\text{mol/l}$)	41 ± 12	54 ± 10	<0.0001
Adolescents creatinine ($\mu\text{mol/l}$)	58 ± 13	60 ± 11	
Total calcium (mmol/l)	2.14 ± 0.31	2.29 ± 0.23	0.0007
Corrected Ca (mmol/l) ^a	2.22 ± 0.31	2.36 ± 0.24	0.0025
Phosphates (mmol/l)	1.36 ± 0.38	1.22 ± 0.23	0.0072
Magnesium (mmol/l)	0.70 ± 0.14	0.83 ± 0.18	<0.0001
Children ALP (IU/l)	453 ± 202	407 ± 123	
Adolescents ALP (IU/l)	596 ± 227	384 ± 136	<0.0001
25-(OH)D (nmol/l)	44 ± 16	36 ± 12	0.0001

Significant differences between boarders and controls are noted (Student's unpaired *t*-test and Mann and Whitney *U* test).

^aSerum values of total calcium concentrations are corrected for protidemia.

Table 3 Clinical characteristics as regards 25-(OH)D levels (mean $\pm$ s.d.) in the boarding school cohort. Significant differences compared to values or prevalence observed with 25-(OH)D values ≤ 30 nmol/l are noted. Statistical tests were χ^2 -test for prevalence and unpaired *t*-test and Mann and Whitney *U* test for age and anthropometry. Sex and pubertal maturation subgroups were too small (<30 individuals) for χ^2 -analyses of prevalence.

25-(OH)D	Up to 30 nmol/l	31–40 nmol/l	41–50 nmol/l	51–82 nmol/l
<i>n</i>	47	67	53	59
25-(OH)D (nmol/l)	26.5 $\pm$ 3.5	35.6 $\pm$ 2.8	45.9 $\pm$ 2.7	65.2 $\pm$ 12.0
Age (years)	11.8 $\pm$ 1.9	11.5 $\pm$ 1.6	11.3 $\pm$ 2.0	11.2 $\pm$ 1.7
Children (<i>n</i>)	9.9 $\pm$ 1.1 (20)	10.2 $\pm$ 0.9 (33)	9.7 $\pm$ 1.2 (29)	10.2 $\pm$ 1.1 (36)
Adolescents (<i>n</i>)	13.2 $\pm$ 1.0 (27)	12.7 $\pm$ 1.0 (34)	13.1 $\pm$ 1.1 (24)	12.9 $\pm$ 0.9 (23)
BMI (z-score)				
Children	−0.10 $\pm$ 0.83	−0.24 $\pm$ 1.00	−0.07 $\pm$ 1.08	0.07 $\pm$ 0.88
Adolescents	−0.18 $\pm$ 1.04	−0.44 $\pm$ 1.30	−0.083 $\pm$ 0.95	−0.64 $\pm$ 1.05
Height (z-score)				
Children	−0.20 $\pm$ 0.91	−0.62 $\pm$ 0.86	−0.50 $\pm$ 0.97	−0.42 $\pm$ 0.87
Adolescents	−0.74 $\pm$ 1.10	−1.04 $\pm$ 1.04	−0.92 $\pm$ 1.46	−1.36 $\pm$ 0.88
Prevalence of				
Genu valgum	36%	9% ^{0.0007}	11% ^{0.0042}	7% ^{0.0002}
Children	40%	18%	21%	8%
Adolescents	33%	0%	0%	4%
Girls	36%	3%	11%	5%
Boys	37%	11%	11%	7%
Hypocalcemic seizure	0%	4%	0%	0%
Respiratory	8%	4%	2%	8%
Bone pain	8%	6%	6%	13%
Scoliosis	16%	7%	11%	12%
Dental caries	8%	9%	13%	8%
Poor dental health	85%	83%	79%	83%
Digestive symptoms	71%	64%	68%	46%
Parasitosis	55%	43%	53%	47%

Bow legs and other features associated with low vitamin D status

To evaluate associations between vitamin D status and clinical, or biochemical, features, boarders were segregated on the basis of their serum 25-(OH)D levels using 30, 40, and 50 nmol/l cutoffs. Boarders with 25-(OH)D levels ≤ 30 nmol/l showed a high prevalence of bow legs (36%), compared with boarders with 25-(OH)D levels between 31 and 40 nmol/l (9%), between 41 and 50 nmol/l (13%), or above 50 nmol/l (7%). This 25-(OH)D-dependent prevalence of genu varum/valgum was not influenced by pubertal status and gender (Table 3). The prevalence of bow legs was too

low in the control cohort (5 children, 7% of the cohort), and the size of the cohort was too small to enable association studies using the 25-(OH)D cutoffs. But none of the 28 controls with 25-(OH)D levels ≤ 30 nmol/l showed lower limb deformities.

Low 25-(OH)D levels (below or equal to 30 nmol/l) were also associated with higher serum alkaline phosphatase activities in prepubertal boarders (Table 4). No such association was observed during puberty, alkaline phosphatase values being markedly high in the adolescent subgroup of boarders, irrespective of their 25-(OH)D levels (Tables 2 and 4). Again, no correlation was observed between serum alkaline

Table 4 Biology as regards 25-(OH)D levels in full-time boarders (mean $\pm$ s.d.). Values significantly different from those observed with 25-(OH)D values higher than 50 nmol/l are noted (using pairwise unpaired *t*-test and Mann and Whitney *U* test).

25-(OH)D (nmol/l)	Up to 30 nmol/l (<i>n</i> = 47)	31–40 nmol/l (<i>n</i> = 67)	41–50 nmol/l (<i>n</i> = 53)	51–82 nmol/l (<i>n</i> = 59)
Proteins (g/l)	70 $\pm$ 10	67 $\pm$ 10	71 $\pm$ 10	69 $\pm$ 12
Hb (g/l)	11.0 $\pm$ 1.1	11.4 $\pm$ 1.0	11.4 $\pm$ 1.0	11.2 $\pm$ 1.1
Glucose (mmol/l)	4.2 $\pm$ 0.8	4.0 $\pm$ 0.8	4.2 $\pm$ 0.8	4.2 $\pm$ 0.8
Children creatinine (μ mol/l)	44 $\pm$ 14	40 $\pm$ 12	41 $\pm$ 12	41 $\pm$ 9
Adolescents creatinine (μ mol/l)	60 $\pm$ 14	56 $\pm$ 13	59 $\pm$ 12	61 $\pm$ 14
Total Ca (mmol/l)	2.10 $\pm$ 0.26 ^{0.0205}	2.12 $\pm$ 0.30 ^{0.0340}	2.11 $\pm$ 0.33 ^{0.0259}	2.24 $\pm$ 0.32
Corrected Ca (mmol/l)	2.15 $\pm$ 0.28 ^{0.0083}	2.22 $\pm$ 0.31	2.16 $\pm$ 0.35 ^{0.0100}	2.32 $\pm$ 0.35
Phosphates (mmol/l)	1.33 $\pm$ 0.34	1.35 $\pm$ 0.41	1.37 $\pm$ 0.38	1.39 $\pm$ 0.37
Magnesium (mmol/l)	0.70 $\pm$ 0.14	0.67 $\pm$ 0.12	0.71 $\pm$ 0.14	0.72 $\pm$ 0.15
Children ALP (IU/l)	534 $\pm$ 148 ^{0.0138}	439 $\pm$ 231	432 $\pm$ 187	437 $\pm$ 211
Adolescents ALP (IU/l)	596 $\pm$ 228	593 $\pm$ 219	633 $\pm$ 244	573 $\pm$ 228

phosphatase and AST (or ALT) transaminase activities, neither before nor during puberty (data not shown).

Finally, serum 25-(OH)D levels were positively associated with the serum calcium levels of boarders ($r=0.202$, $P=0.0024$): boarders with 25-(OH)D levels ≤ 30 nmol/l, and even those with intermediate levels (30–50 nmol/l), had lower serum calcium levels than boarders with 25-(OH)D levels > 50 nmol/l (Table 4). In contrast, serum calcium was not associated with 25-(OH)D levels in the control cohort, and boarders had significantly lower serum calcium than controls when their 25-(OH)D levels were ≤ 30 nmol/l (2.15 ± 0.28 vs 2.41 ± 0.16 mmol/l, $P<0.0001$), but not when their 25-(OH)D levels were above 50 nmol/l (2.32 ± 0.35 vs 2.50 ± 0.12 mmol/l).

Discussion

Moderate leg bowing may be regarded as physiological in young children, aged < 2 –5 years, with first a varus followed by a valgus angulation at the knee joint (31). But this feature is transient in white North American (27), Nigerian (32), and Asian (33, 34) children, and bowing of the lower extremities is considered to be abnormal after the age of 7–8 years (32–34). Furthermore, $< 2.5\%$ of 196 North American white children, aged 6 months to 11 years, had knock-knees with an IM distance above 8 cm (27). Likewise, a European study of 427 adolescents aged 10–16 years showed a mean IM of 0–2 cm, with a prevalence lower than 2.5% of IC above 4–5 cm, and of IM above 4 cm in boys and 8 cm in girls (28). These observations contrast with the present findings of a high prevalence (15%) of genu varum/genu valgum (IC or IM distances above 5 cm) in a homogeneous cohort of 226 white European children and adolescents living in a Moldovan boarding school.

Abnormal leg bowing may result from rare diseases like hereditary rickets, osteogenesis imperfecta, or achondroplasia, but its pathogenesis habitually appears multifactorial, possibly involving genetic factors and several environmental contributors that are often associated: increased skin pigmentation, insufficient exposure to sunshine, atmospheric pollution, high fluoride content of the drinking water, low milk/dairy product intakes and/or calcium deficiency, and high fiber and/or low meat intakes (11, 18–24).

Low vitamin D status likely contributed to the genesis of lower limb deformities in the present cohort, as bow legs or knock-knees were found in 36% of the full-time boarders seen during winter–spring with 25-(OH)D levels ≤ 30 nmol/l, whatever their sex or pubertal maturation, and only in 10% of those with 25-(OH)D levels in the intermediate 30–50 nmol/l range, and in 7% of those with higher levels.

The 25-(OH)D status was also associated with markers of calcium and bone metabolism, namely serum

alkaline phosphatase and serum calcium. Prepubertal children with 25-(OH)D levels ≤ 30 nmol/l had increased serum alkaline phosphatase activities, with no simultaneous increase in transaminase activity, thus suggesting underlying rickets even though this could not be confirmed because bone X-rays had not been performed for ethical reasons. No such association was observed in adolescent boarders, but it should be noted that their serum alkaline phosphatase activities, irrespective of their serum 25-(OH)D levels, were in the range of the prepubertal boarders with a low vitamin D status, which was higher than that measured in age-matched controls. The lack of association with serum transaminase activities suggests that the high alkaline phosphatase activities found in boarders during puberty reflect an increased bone turnover, possibly due to calcium deficiency. But measurements of serum PTH, bone resorption markers, and/or bone mineral densitometry are clearly needed to test this hypothesis. Finally, 25-(OH)D levels were positively associated with serum calcium levels, with optimal 25-(OH)D levels above 50 nmol/l. No association was found with calcium-dependent clinical symptoms, hypocalcemic fits especially, probably because boarders were older and/or because their hypocalcemia and vitamin D deficiency were moderate, compared to previously reported cases (26, 35). Vitamin D status varies with the season in temperate countries, being lower during winter–spring when the u.v. energy in the solar light becomes insufficient for the skin production of vitamin D. The present association study unmasks a possibly higher risk for low vitamin D status in girls and shows that a low 25-(OH)D status level, whether or not only in winter–spring, clearly aggravates the prevalence of lower limb deformities and of low serum calcium in children and adolescents of European origin.

Yet low vitamin D status was not the sole contributor to the high prevalence of lower limb deformities and to the lower serum calcium levels observed in the boarding school. First, the full-time boarders living in a rural setting had a vitamin D status higher, between January and April, than Moldovan controls living in Chisinau city. Second, the 22% incidence of 25-(OH)D values ≤ 30 nmol/l observed during winter–spring in the boarding school was comparable to that reported in other European countries (17–35%) located at similar latitudes (46° – 51° N), namely France, Germany, and Switzerland (1–3). Third, the prevalence of lower limb deformities in boarders with 25-(OH)D values ≤ 30 nmol/l was markedly higher (36 vs 0%), and the serum levels of calcium were significantly lower, than in white Moldovan controls with a similarly low 25-(OH)D status. Digestive disorders or parasitic infections may have aggravated the risk of lower limb deformities, but their prevalence, similar in the two Moldovan cohorts, cannot *per se* explain the anomalies found in the boarding school. Fluoride intoxication has not been evaluated, but cannot be excluded as an additional

contributor, as suggested by reports of children with rachitic-like bone deformities in regions of endemic fluorosis (11, 18, 21) and observations of high fluoride levels in potable water in some Moldovan districts (36). But a major contributor was likely the cereal-based diet with little access to meat, milk, and dairy products, an unusual diet for Moldovan children, including the urban controls, who theoretically receive adequate calcium/milk intakes (37, 38). In agreement with this hypothesis, low calcium/milk intakes have been associated with rickets, even in the absence of severe vitamin D deficiency, in some regions of Nigeria and Bangladesh (11, 23), and low meat and high fiber intakes have been associated with an increased rachitic and osteomalacic relative risk in the UK (39).

In conclusion, there is an emerging evidence that a very low calcium intake may contribute to the etiology of rickets in Africa and Asia (23), and that calcium deficiency may lower the threshold under which vitamin D status becomes insufficient to ensure the optimal mineralization of the growing skeleton in children (11) and adolescents (2). The present findings show that lower limb deformities and low serum calcium may be frequent in subsets of Caucasian children and adolescents and highlight the major impact of combined calcium/milk and vitamin D deficiency on bone mineralization during pre-pubertal and pubertal growth. In the presence of risk factors like low calcium/milk intakes, possibly aggravated by intestinal malabsorption and parasitosis, optimal 25-(OH)D levels for bone health and calcium metabolism appear to be higher than 30 nmol/l, and possibly higher than 50 nmol/l, in agreement with thresholds proposed earlier for optimal bone mass accrual during growth (2, 6, 8, 16, 40). Because of the high prevalence of low 25-(OH)D levels (≤ 30 nmol/l) during winter–spring, dietary fortification, or supplementation with vitamin D, may be recommended, at least during the winter, to European children and adolescents with no or insufficient calcium/dairy product intakes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M Garabedian, L Esterle, and A Voloc conceived and designed the experiments. A Voloc recruited the cohort and clinically examined the children. L Esterle, T M Nguyen, O Walrant-Debray, and A Colofitchi performed the experiments. L Esterle and M Garabedian analyzed the data. L Esterle and M Garabedian wrote the paper.

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