Clinical Nutrition 38 (2019) 912–919

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Vitamin D nutritional status and bone turnover markers in childhood acute lymphoblastic leukemia survivors: A PETALE study

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ARTICLE INFO

Article history:
Received 23 October 2017
Accepted 3 February 2018

Keywords:
Acute lymphoblastic leukemia
25-Hydroxyvitamin D_3
Bone resorption
Bone formation
CTX
P1NP

SUMMARY

Background: The remarkable progress in the treatment of childhood acute lymphoblastic leukemia (cALL) has led to a survival rate reaching 90%. This success story is unfortunately linked to increased risk of impaired skeletal mass accumulation during childhood and adolescence, predisposing the patients to osteoporosis and pathological fractures at adulthood.
Objective: This study aims at characterizing the vitamin D status and bone health biomarkers in a well-characterized cohort of cALL survivors.
Results: Food frequency questionnaires reveal that (i) the total vitamin D intake varies greatly (44–2132 IU/d), (ii) only 16.8% of the participants consume vitamin D supplements, and (iii) 74% of survivors' intakes are below the Recommended Daily Intakes (400 IU/d). For the 42 participants taking vitamin D supplements, the median (2.5–97.5$^{\%iles}$) intake is 600 IU/d (21.2–1972 IU/d). Sixteen participants are vitamin D deficient (<30 nM) and 66 insufficient (≥30 − <50 nM). Serum 24,25$(OH)_2D_3$ concentrations are directly related to those of 25OHD_3, and those of 3-epi-25OHD_3 below the Lower Limit of Quantification in most samples. The participants' serum concentrations of cross-linked C-telopeptide of type-I collagen and intact amino-terminal pro-peptide of type-I collagen decrease steadily with age, leveling at adulthood, and are at all times higher in males.
Conclusion: The present study shows that the prevalence of vitamin D insufficiency or deficiency is not greater in cALL survivors compared to the general Canadian population despite low vitamin D food and supplement intakes. Furthermore, there seem to be no overt imbalance in the gender- and age-adjusted serum bone turnover marker concentrations.

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https://doi.org/10.1016/j.clnu.2018.02.006
0261-5614/

What is known?

Childhood ALL (cALL) surviving patients are at risk of developing secondary bone pathologies.
cALL surviving patients are at risk of suffering from vitamin D insufficiency or deficiency.

What does this paper add?

Information about well characterized cALL survivors vitamin D and calcium nutritional status.
Knowledge on cALL survivors' vitamin D profile.
Insight into calls survivors' bone turnover markers' status.
Uncovering the correlation between the vitamin D nutritional status and bone turnover markers.

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. As its name indicates, it involves a blocking in the hematopoietic stem cell differentiation process [1]. The remarkable progress in the treatment of childhood ALL (cALL), based on risk stratification and improved clinical support, has led during the last two decades to a survival rate reaching 90% [2,3]. This success story is unfortunately however linked to risks of secondary chronic diseases including obesity and cardiometabolic complications [4–6]. The treatment protocol, as well as survivors' lifestyle and genetic predisposition have all been invoked as potential causes of these metabolic impairments [4]. On another front, skeletal changes such as metaphyseal bands, periosteal reaction, osteolysis, sclerosis and osteoporosis resulting in vertebral compression, observed at the time of diagnosis have been attributed to the disease process *per se* [7–12]. In addition the intense treatment protocols including methotrexate and glucocorticoids are considered aggravating factors in the abnormal accumulation of skeletal mass during childhood and adolescence, predisposing these patients to osteoporosis and pathological fractures in early adulthood [13–16]. Osteonecrosis, with the potential of affecting joint functionality is also a long-term post-treatment complication in cALL survivors [17]. These debilitating conditions have in common the failure of attaining an optimal bone mass during adolescence [18,19].

Vitamin D, through its hormonal metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$) [20], is a key factor influencing bone mass accretion. Its largely described central role resides in modulating gene expression in bone cell differentiation needed for balanced bone mineralization [21,22]. More specifically, $1,25(OH)_2D$ has been shown to inhibit the rate of human primary osteoblast proliferation and to enhance the expression of the vitamin D-responsive osteocalcin (*BGLAP*), bone sialoprotein-1 (*SPP1*) and intracellular adhesion molecule 1 (*ICAM1*) genes [22,23]. Although the kidney appears as the dominant site for the conversion of 25OHD to $1,25(OH)_2D$ via the mitochondrial $25OHD_3$ 1α-hydroxylase (CYP27B1) [24], mounting evidence indicate that this important enzyme is widely distributed peripherally. It is present, together with the vitamin D nuclear receptor (VDR), in osteoblasts, osteocytes, chondrocytes and osteoclasts [22,25,26]. The required machinery present in those bone cells allows complementary paracrine and autocrine activities of $1,25(OH)_2D$ [20,22,25]. These observations bear an important clinical significance as they provide an explanation for the higher need in circulating 25OHD to prevent fractures than for the normalization of intestinal calcium absorption and maintenance of normal blood calcium homeostasis. Indeed, clinical studies have shown that marked secondary hyperparathyroidism occurs only when serum 25OHD concentrations fall below the 20 nmol/L threshold [27–29].

There is a perception that cALL survivors are at increased risk for vitamin D deficiency, explained in part by sun exposure avoidance during hospitalization and lengthy convalescence, and by nutritional issues. Decreased vitamin D bioavailability due to chemotherapy-induced gut mucositis [30], and increased catabolism due to glucocorticoid immunosuppressive treatment represent aggravating factors [31]. Simmons et al. [32] reported that 53% of cALL survivors suffered from vitamin D insufficiency, defined as serum 25OHD concentrations between 37.5 and 50 nmol/L, and 12% of vitamin D deficiency (<37.5 nmol/L). A year later, Modan-Moses et al. [33] using the same thresholds confirmed these data by showing that 25% of pediatric patients with different forms of cancers suffered from vitamin D deficiency and 23% from insufficiency.

The present study primarily aims at assessing the vitamin D status of cALL survivors, using the 2016 expert consensus recommendations on the classification of vitamin D nutritional status [34] taking into account potential modifiers such as body composition and year period for sampling. The secondary objective is to assess the impact of the vitamin D status on the bone resorption and formation biomarkers via serum cross-linked C-telopeptide of type I collagen (CTX) and intact amino-terminal pro-peptide of type I collagen (iP1NP) respectively. This bears importance for improving prevention strategies in at-risk populations.

2. Material and methods

2.1. Patients

The study group, part of the PETALE cohort, consists of 251 French-Canadian cALL survivors of European origin [35,36], 124 males and 127 females aged between 8.5 and 41 years, living between the 45th and 49th parallel. They were diagnosed and treated at the SJUHC (Montreal, Canada) between January 1989 and July 2005 [37,38]. The median age ($2.5–97.5^{\%ile}$) at diagnosis was 4.8 (1.5–17.1 years, and all treated with the Dana Farber Cancer Institute protocols [39]. The median treatment time ($2.5–97.5^{\%ile}$) was 2.1 (1.4–2.4) and the period post-treatment was 13.0 (4.4–23.8) years. The SJUHC Institutional Review Board of Sainte-Justine Hospital approved the study and the investigations were carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from study participants and/or parents/guardians.

2.2. Anthropometric measurements

For children and adolescents, overweight was defined as a body mass index (BMI) (kg/m^2) ranging between the 85th percentile and the 95th age- and sex-specific percentile of the Centers for Disease Control and Prevention 2000 growth charts, and obesity was defined as BMI >95th percentile [40,41]. For adult participants, those with a BMI >25 and ≤30 kg/m^2 were considered overweight, and those with a BMI >30 kg/m^2 as obese. For adults, waist circumference was classified as normal (<94 cm in men and <80 cm in women), borderline (≥94 cm and <102 cm in men and ≥80 cm and <88 cm in women) [42] or high (≥102 cm in men and ≥88 cm in women) [43]. For children, waist circumference below the 90^{th} percentile was defined as normal, ≥90^{th} and <95th percentile as borderline and ≥95th percentile as high [44,45].

2.3. Nutritional assessment

A registered nutritionist administered a food-frequency questionnaire that was developed and validated specifically for a French speaking population of children adolescent and young adults. Patients were also asked to report supplements to evaluate total calcium, phosphorus and vitamin D intakes. Evaluation of nutrient intakes was performed using the Canadian Nutrient File Database (www.healthcanada.gc.ca/cnf).

2.4. Biochemical assays

Total calcium (Ca_t), inorganic phosphate (P_i) and alkaline phosphatase (Alk Phos) were measured on an Architect *c*Systems (Abbott Diagnostics, Mississauga, ON, Canada). Their respective intra-assay coefficients of variation were: (1.01% at 1.60 mmol/L and 0.60% at 3.42 mmol/L), (0.70% at 0.74 mmol/L and 0.61% at 2.38 mmol/L), (1.79% at 32 IU/L and 0.40% at 337 IU/L); their inter-assay coefficients of variations were: (1.3% at 1.47 mmol/L and 1.0% at 3.31 mmol/L), (3.17% at 0.63 mmol/L and 1.72% at 2.33 mmol/L), (5.0% at 31 U/L and 2.0% at 321 U/L). Intact Parathyroid hormone (iPTH) was measured on an Immulite 2000 (Siemens Canada Ltd., Oakville, ON). The inter-assay variations were 9.8% at 3.2 pM, 6.2% at 30.5 pM and 5.7% at 105 pM. iP1NP and CTX were measured by two-site chemiluminometric immunoassays on the IDS-iSYS automated analyzer (Immunodiagnostic Systems, Tyne & Wear, UK) at the Montreal Shriners Hospital for Children. For P1NP, the intra-assay imprecision was 4.6%, 4.0% and 4.8% at respective iP1NP concentrations of 30.6, 105.2 and 187.6 μg/L. The between batch imprecision was 8.1, 7.6 and 6.0% at 31.8, 109.4 and 1195.1 μg/L respectively; for CTX the intra-assay imprecision was 2.8, 1.7 and 2.3% at 0.171, 1.046 and 1.718 μg/L and inter-assay imprecision was 5.5, 3.3 and 3.5 at 0.173, 1.065 and 1.736 μg/L. The inter- and intra-assay precisions were evaluated in accordance with the Clinical Laboratory Standards Institute (CLSI) guideline EP-5A2 [46]. Professor Étienne Cavalier generously provided the reference values for serum P1NP and CTX obtained from Belgian children, adolescents and adults, measured on the IDS-iSYS automated analytical platform. From these reference values, we calculated the age- and sex-adjusted Z-Scores and derived a "bone turnover index" by subtracting CTX Z-scores from P1NP Z-scores. Under those conditions positive values favor bone formation and negative values resorption.

2.5. Vitamin D profiling

Vitamin D metabolite profiling was performed with an in-house stable isotope-dilution quadrupole time-of-flight mass spectrometry method (MS-QTOF) (Waters UPLC-MS system (Xevo G2 quadrupole time-of-flight, Waters Corp., Mississauga, Ont.). The method based on that of Kaufmann et al. [47] is described in the companion paper [48]. The intra- and inter-assay coefficients of variation were respectively 4.2 and 7.0% at 70 nM $25OHD_3$, 5.1 and 10% at 15 nM $24,25(OH)_2D_3$ and 4.5, 8.9% at 20 nM 3-epi-$25OHD_3$ and 7.0, 10.2% for $25OHD_2$. The lower limits of quantification (LLoQ) for $25OHD_3$, $24,25(OH)_2D_3$, 3-epi-$25OHD_3$ and $25OHD_2$ were respectively 2.5, 2.0, 2.0 and 2.5 nM. The following classification of vitamin D status based on serum 25OHD concentrations necessary for preventing rickets, and on the consensus report of experts, is as follows: sufficiency: >50 nM; insufficiency: ≥30–<50 nM; and deficiency: <30 nM [34].

2.6. Statistics

Descriptive statistics were used to report PETALE cALL survivors' characteristics according to gender. Data are expressed as mean and standard deviation for normally distributed variables and as median and inter-quartile range (IQR) for those that are not. The associations between serum $25OHD_3$ and predictors (vitamin D intake, percent body fat, serum iPTH, BMI and waist circumference) were tested by simple regression analysis. The Kruskal–Wallis followed by the two-stage linear step-up procedure of Benjamini, Krieger, Yekutieli for controlling false discovery rate were used to verify the year-period variations in serum $25OHD_3$ concentrations. A $P \leq 0.05$ is considered significant. Statistical analyses were performed with GraphPad Prism 7.0a (GraphPad Software Inc., La Jolla, CA) and SAS 9.4 (SAS Institute Inc., Cary, NC).

3. Results

Demographic, anthropometric and classical biochemical characteristics according to gender of the participants are shown in Table 1. The age at entry into the study, the number of years of treatment and the period after the end of treatment do not differ between male and female participants. The median BMI (IQR) is similar for both sexes and there is no difference in the distribution according to lean, overweight and obese classification (χ^2 = NS).

The food frequency questionnaires reveals that median (IQR) total vitamin D intake varies greatly, and is higher in males than females [280 (200–412) vs 224 (160–376) IU/d, $P = 0.0103$]. We also find that only 16.8% of the participants (both males and females) consume vitamin D supplements, and that 74% of them are below the RDI (400 IU/d) [49]. For those who take supplements

Table 1
Characteristics of participants.

Characteristics	Males (n = 124)	Females (n = 127)	P[c]
Age at entry (Years)	22.4 (17.1–26.4)	21.9 (16.8–26.1)	
Distribution [%]			
<20	41.9	40.9	NS
≥20–<30	47.6	46.5	
≥30–41	10.5	12.6	
[a]Years of treatment	2.1 (2.0–2.2)	2.1 (2.1–2.2)	NS
[a]Years after treatment	13.1 (9.8–17.1)	13 (9.3–18.9)	NS
[a]BMI [kg/m^2]	23.0 (21.0–26.2)	23.7 (20.8–26.5)	NS
BMI category [%]			
Lean	72.6	69.3	NS
Overweight	14.5	14.2	
Obese	12.9	16.5	
[ad]Total Vitamin D intake [IU/d]	280 (200–412)	224 (160–376)	=0.0103
[a]Calcium intake [mg/d]	1627 (1156–2060)	1235 (947–1526)	<0.001
[a]Phosphate intake [mg/d]	2105 (1689–2555)	1619 (1330–1962)	<0.001
[b]Serum Calcium [mM]	2.44 ± 0.08	2.38 ± 0.08	<0.001
[b]Serum Inorganic Phosphate [mM]	1.25 ± 0.28	1.24 ± 0.19	NS
[a]Serum Alkaline Phosphatase [IU/L]	80 (66–111)	66 (50–87)	<0.001
[a]Serum iPTH [pM]	3.2 (2.2–4.1)	3.1 (2.1–4.0)	NS
[a]Serum $25OHD_3$ [nM]	57 (42–72)	64 (46–80)	=0.0403

The current laboratory-derived reference values are: serum calcium (1–19 years: 2.29–2.63 mM; >19 years: 2.10–2.55 mM); inorganic phosphate (Males 13–16 years: 1.14–1.99; Females 13–16 years: 1.02–1.79; Males & Females 16–19 years: 0.95–1.62; Males and Females >19 years: 0.74–1.52 mM); serum total alkaline phosphatase (Males 13–15 years: 127–517 IU/L; Females 13–15 years: 62–280 IU/L; Males 15–17 years: 89–365 IU/L; Females 15–17 years: 54–128 IU/L; Males 17–19 years: 59–164 IU/L; Females 17–19 years: 48–95; Males and Females >19 years: 40–150 IU/L). The serum iPTH reference values are those suggested by the manufacturer (1.3–6.8 pM).

[a] Median (IQR).

[b] Mean (SD).

[c] P values from t-tests for continuous variables and chi^2 tests for categorical variables.

[d] To convert IU/d vitamin D intake to μg/d divide by 40.

(n = 42), the median (IQR) intake is 600 IU/d (IQR: 356–1052). Similarly to vitamin D, males have higher daily calcium intakes than females [1627 (1156–2060) vs 1235 (947–1526) mg/d, $P < 0.001$], with only 14 total participants (5.6%) being below the RDI (800 mg/d). Phosphate intakes follow those of calcium.

Serum total calcium and inorganic phosphate are all within normal values although male participants exhibit marginally but statistically significant higher serum calcium concentrations than females (2.44 ± 0.08 vs 2.38 ± 0.08, $P < 0.001$). Serum total alkaline phosphatase activities are all within age- and sex-adjusted normal values but are statistically higher in males than females ($P < 0.001$). The circulating iPTH median (IQR) concentrations are within normal ranges and do not differ between male and female participants.

Despite higher vitamin D intakes in males, their serum $25OHD_3$ concentrations [median (IQR)] are lower than in females [57 (42–72) vs 64 (46–80) nM, $P = 0.0403$], with 9 male and 6 female participants being deficient (<30 nM). The serum $24,25(OH)_2D_3$ concentration ranges from 2 to 29 nM (median: 5.5) and is directly related to the $25OHD_3$ concentrations. 3-epi-$25OHD_3$ concentrations are below the LLoQ in most samples. For those in whom it is quantifiable (n = 55), the median (IQR) is 3.1 (2.5–3.8) nM.

The year quarter during which sampling is performed is also a known determinant of serum $25OHD_3$ concentrations (Fig. 1). As expected, median concentrations observed for the 3rd & 4th quarters (July–September & October–December) are statistically higher than those for the 1st and 2nd (January–March & April–June), reflecting the vitamin D reserve obtained during the higher solar irradiation period. Table 2 shows that obesity, defined by high BMI, is a negative predictor for serum $25OHD_3$ concentrations [(β: −11.1035 (C.I.: −20.2925 to −1.9145, $P = 0.0181$)], but that percent body fat and waist circumference are not. Figure 2 depicts the inverse function between serum $25OHD_3$ and iPTH concentrations.

Table 2
Association between $25OHD_3$ and vitamin D intake, body fat, BMI and waist circumference.

	Regression coefficient estimation	95% CI		*P*-value
Vitamin D intake	0.5384	0.2039	0.8750	0.0017
Body fat %	−0.1018	−0.3779	0.1743	0.4682
BMI				
Overweight vs normal	−1.1989	−8.7191	6.3213	0.7538
Obesity vs normal	−11.1035	−20.2925	−1.9145	0.0181
Waist circumference				
Overweight vs normal	−2.0432	−11.2519	7.1654	0.6625
Obesity vs normal	−4.1462	−11.2243	2.9320	0.2497

The associations between serum $25OHD_3$ and continuous variables (vitamin D intake and percent body fat) were tested by univariate regression analysis. Chi-squared tests were performed for categorical classification (BMI and Waist circumference). A $P \leq 0.05$ is considered significant.

Table 3 compares the concentrations of the bone resorption (CTX) and formation (P1NP) biomarkers for participants in this study to a reference group consisting of healthy European volunteers. As can be appreciated, the concentrations of both bone turnover biomarkers decrease steadily with age, leveling at adulthood, and are at all times higher in males than in females. The number of cALL survivors and of referent individuals in each age categories being limited, we are unable to state whether participants' values are within the range of the reference group or not.

Table 4 shows the adjusted effect of gender, age, vitamin D status, BMI, waist circumference and body fat on serum P1NP and CTX concentrations. As the P1NP and CTX data are not normally distributed, the values are log-transformed. As can be appreciated gender, age, percent body fat and vitamin D status are determinants

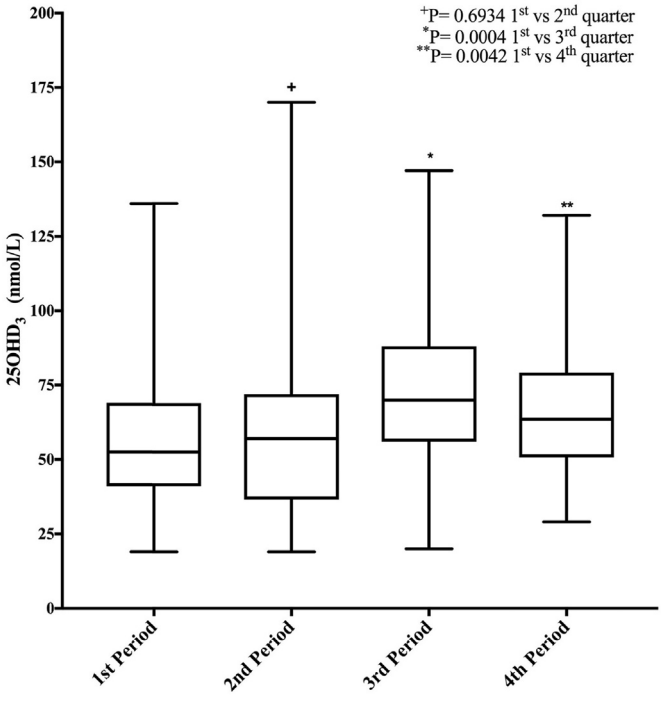

Fig. 1. Serum $25OHD_3$ concentrations by year quarters. $25OHD_3$: 25-hydroxyvitamin D_3. The one-way ANOVA Kruskal–Wallis rank test, followed by the two-stage linear step-up procedure of Benjamini, Krieger, Yekutieli for controlling false discovery rate was used. The 1rt period served as reference. The box and whisker plots show the medians, interquartile ranges and minimum and maximum concentrations. The 1st period covers: January–March (n = 68), the 2nd: April–June (n = 81), the 3rd: July–September (n = 37), the 4th: October–December (n = 62).

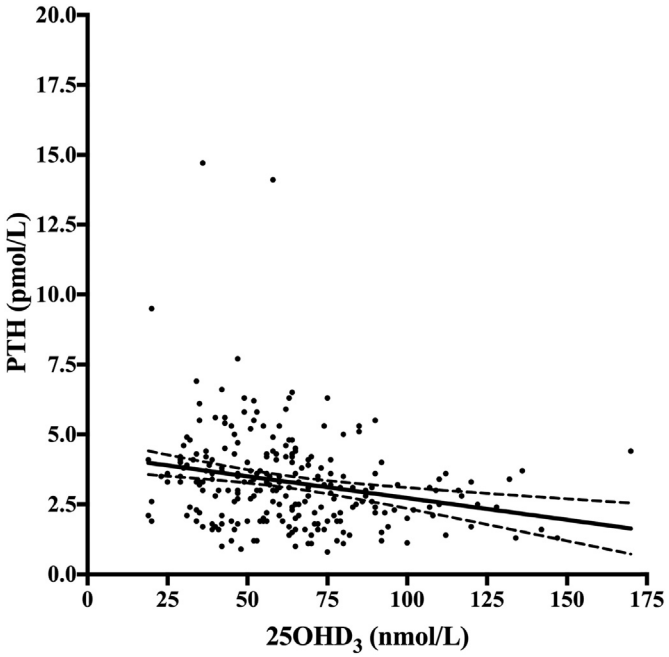

Fig. 2. Relationship between serum $25OHD_3$ and iPTH concentrations. iPTH: intact Parathyroid hormone; Parathyroid hormone; $25OHD_3$: 25-hydroxyvitamin D_3. A Linear regression was used. The dotted lines represent the 95% confidence intervals (C.I.). For this analysis 244 paired samples were available. Equation: Y = −0.01555*X (−0.0238 to −0.0073) + 4.284; $P = 0.0003$. Values in parentheses are the 95% C.I.

Table 3
Patient serum bone resorption and formation biomarkers concentrations.

Males	n	Patients	Control values	Patients	Control values
Age group (y)		CTX μg/L	CTX μg/L	P1NP μg/L	P1NP μg/L
8–<10	1	2.33	0.78–2.55	639	402–968
10–<12	1	3.75	0.77–2.58	1119	300–1190
12–<14	3	2.17–3.59	0.61–2.60	926–1156	146–1192
14–<16	10	1.37–4.15	0.53–3.05	259–1849	139–1030
16–<18	26	0.75–4.34	0.29–1.83	92–619	61–608
18–<20	11	0.53–1.86	0.20–1.56	60–474	27–364
>20	71	0.03–1.46	0.02–0.80	26–228	11–79
Females	n	Patients	Control values	Patients	Control values
Age group (y)		CTX μg/L	CTX μg/L	P1NP μg/L	P1NP μg/L
8–<10	0	–	0.32–2.52	–	181–1337
10–<12	2	0.25–0.81	0.44–2.14	421–851	382–1312
12–<14	3	0.39–1.20	0.17–1.72	230–1081	74–713
14–<16	19	0.13–2.31	0.53–2.51	68–991	50–342
16–<18	19	0.10–2.10	0.32–1.53	28–264	16–162
18–<20	9	0.19–0.57	0.12–1.50	45–145	19–147
>20	75	0.07–2.16	0.03–0.66	2–120	7–57

Patient values are compared to reference values. When the number of patients allows, values are expressed as the 2.5–97.5th percentiles. Otherwise they are the range. The reference values were obtained from groups of volunteer European pediatric-age male and female (n = 23–25), premenopausal women (n = 124) and middle-age men (n = 138). The 2 biomarkers were measured on the same platform as that used in the present study.

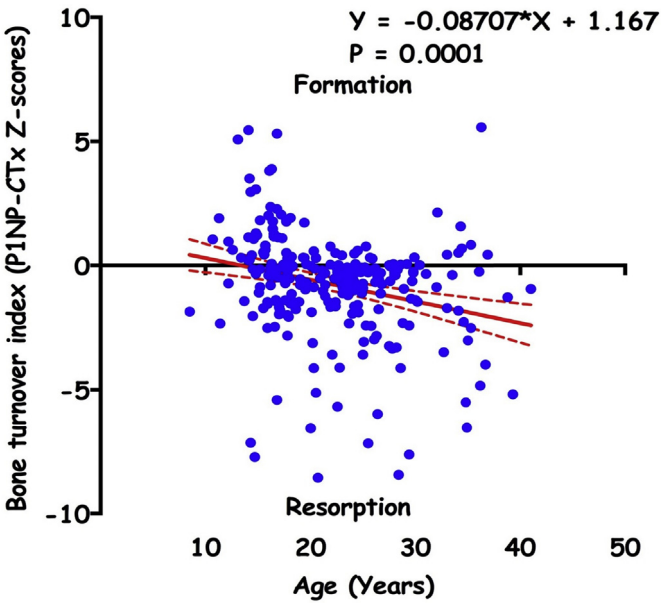

Fig. 3. Bone turnover index as a function of age of the cALL survivors. P1NP: propeptide of type I collagen; CTX: cross-linked C-telopeptide of type I collagen; linear regression was applied. The equation for the regression is: Y = −0.08707 (−0.1285 to −0.04564) X + 1.167, r^2 is 0.06665; $P < 0.0001$. The values in parentheses are the slope 95% Confidence intervals (C.I.); N = 242.

of the concentrations of both bone turnover biomarkers. Interestingly, waist circumference positively predicts P1NP concentration only when comparing obese and normal subjects. Finally BMI is not a predictor for the bone turnover markers.

Figure 3 depicts the relationship between the age of participants and the bone turnover index described in the Methods section. It can be appreciated that the index shifts from a formation- to a resorption-leading environment.

4. Discussion

This study provides comprehensive data on the vitamin D nutritional status and bone turnover markers for a well-characterized cohort of cALL survivors.

4.1. Nutritional status

Nutritional assessment reveals that whereas three quarters of the patients have total vitamin D intake below the RDI (400 IU/d), a large proportion have a calcium intake above the RDI [49]. The relatively low vitamin D intake is somewhat unexpected, as our *a priori* hypothesis was that these patients would be more inclined to protect themselves, having experienced cALL-related as well as iatrogenic bone diseases. Our nutrition data resemble those of other studies conducted in the Canadian population. A study conducted 10 years ago in 209 Aboriginal and 146 non-Aboriginal women concludes that 61% of urban-dwelling white women aged between 25 and 50 years (n = 87) have adequate intakes (AI) of vitamin D, defined as 200 IU/d [50]. If we were to set the AI at the same level, we would observe a similar percentage for our cohort. In a population-based case control study, having breast cancer risk as primary outcome, Anderson et al. [51] reported that 57% of the participants had vitamin D intakes below the RDI, a value somewhat lower than ours. On the other hand we observe a similar prevalence of inadequate vitamin D intake (below 400 IU/d) in our cohort to that reported by Zhou et al. [52] for women and men 25 years and older. Furthermore in contrast to the vitamin D intake, we observe a low percentage of participants with serum $25OHD_3$ concentrations below the Institute of Medicine threshold (40 nmol/L). Therefore, contrary to our initial working hypothesis the prevalence of vitamin D insufficiency or deficiency is not greater in the PETALE patients than in the general Canadian population [53,54],

Table 4
Multiple linear regression analyses for predictors of bone turnover biomarkers.

	P1NP[a]				CTX[a]			
	Regression coefficient	95% CI		*P*-value	Regression coefficient	95% CI		*P*-value
Sex (Male vs Female)	0.3748	0.1291	0.6205	0.0029	0.4125	0.1907	0.6343	0.0003
Age	−0.0984	−0.1121	−0.0848	<0.0001	−0.0701	−0.0824	−0.0577	<0.0001
$25OHD_3$	−0.0040	−0.0073	−0.0007	0.0177	−0.0039	−0.0069	−0.0009	0.0103
BMI								
Overweight vs normal	0.0473	−0.2083	0.3028	0.7159	0.0922	−0.1385	0.3229	0.4318
Obesity vs normal	−0.1075	−0.4669	0.2519	0.5561	−0.0929	−0.4173	0.2315	0.5732
Waist circumference								
Overweight vs normal	0.0721	−0.2008	0.3450	0.6031	−0.2083	−0.4546	0.0381	0.0971
Obesity vs normal	0.5143	0.2132	0.8154	0.0009	0.1589	−0.1129	0.4306	0.2505
Percentage body fat	−0.0264	−0.0412	−0.0116	0.0005	−0.0191	−0.0325	−0.0057	0.0053

[a] As the concentrations of the intact amino-terminal pro-peptide of type I collagen (iP1NP) and CTX were not normally distributed, the data were log transformed prior to analysis.

with 26.4% and 6.4% of the participants being respectively vitamin D-insufficient (25OHD ≥30 − <50 nM) and −deficient (25OHD <30 nM). As already mentioned we based the classification of the vitamin D nutritional status on needs required for an adequate bone health. However some reports have proposed a serum 25OHD cut-point of 75 nM for optimal health [55,56]. Should this value be used in this study, close to 75% of the participants would then be classified as insufficient, this percentage being similar to the general Canadian population [54]. Although our results appear to differ from those reported for a similar size study conducted in young cancer patients in which 24.6% were vitamin D-deficient, the different threshold for defining vitamin D deficiency (<37.5 nM) explains in part this difference [33]. The inverse relationship between circulating iPTH concentration and serum $25OHD_3$ has been reported by others [27,28,57,58], and will not be further discussed.

The marginally but statistically significant lower serum $25OHD_3$ in males despite higher vitamin D intake is puzzling. The year period can be excluded, as there is no difference in the proportion of samples taken in different year quarter between males and females. We show that BMI is a predictor for serum $25OHD_3$ concentrations (Table 2). However BMI being not different between males and females as shown in Table 1, it does not account for the difference in serum $25OHD_3$ concentrations. A self-reported vitamin D intake at a time close to blood sampling that is not representative of the cumulative vitamin D intake of an extended time could also explain the contradiction. Finally the apparent variance could be due to chance, and be within the limits of uncertainty of the food frequency questionnaires and $25OHD_3$ assay methods.

We show, as others did [59–61], that adiposity expressed as BMI is an explanatory variable for serum $25OHD_3$ concentrations. This particularly holds when comparing the obese group to the lean group. Although we observe a trend for an inverse relationship between serum $25OHD_3$ and percent body fat or waist circumference, it did not reach significance. This is apparently contrary to the observations of Hannemann et al. [62] who reported, in large epidemiological study with more than 7000 men and women, that waist circumference and percent body fat are, in age- and sex-adjusted linear regression models, inversely related to serum $25OHD_3$ concentrations. The small sample size of our cohort associated with wide confidence intervals could explain the differing results.

4.2. Bone turnover markers

As expected, age and sex are the two main explanatory variables for serum concentrations of bone turnover biomarkers. This is particularly striking for children and adolescents, although the dependence on sex is observed even for adults. The values observed are within the range of those provided by Professor Cavalier and those published by others on different analytical platforms and with population of different genetic background [63–66]. In our cohort we observe a modest yet significant negative relationship between serum $25OHD_3$ concentrations and iP1NP and CTX (Table 4). Such data are scarce, in particular in populations at risk such as the subjects of this study. Our results differ from those of Madar et al. [67] who showed, in a 16-week supplementation controlled randomized clinical trial conducted with 251 healthy participants aged between 18 and 50, that increasing serum 25OHD from 75 to 125 nmol/L did not affect the serum bone markers concentrations. It may be that the time frame of this study was not sufficiently long to allow bone metabolic changes that could be translated into modifications of serum bone turnover marker concentrations. Furthermore, contrary to our cohort, all participants had serum 25OHD values exceeding the 75 nmol/L threshold. Ginty et al. [68], in a group of adolescents aged between 11.4 and 16.4 years, also reported that serum 25OHD was not a major determinant of serum P1NP and CTX concentrations. The authors concluded that the pubertal status primarily influenced the bone turnover markers and thus obscured the effect of vitamin D status. However, Thiering et al. [69] in a population based study involving 2798 ten-year old children, observed a clear seasonal variation in serum bone turnover markers concentrations and a negative association between serum 25OHD and serum CTX. With respect to adiposity, although we observe no relationship between BMI and the 2 bone turnover markers, we note that waist circumference, a visceral adiposity surrogate marker, is a strong positive predictor for both bone biomarkers. Surprisingly, the opposite is noticed for percent body fat. Our observations are at variance with those of Tonks et al. [70] who reported, in an observational study involving 61 overweight/obese individuals, increased visceral adiposity associated with lower fasting serum osteocalcin (a bone formation marker) and CTX.

The “bone turnover index” we developed gives additional information on the trajectory of bone metabolism. As such data have, to our knowledge, not previously been published, we are unable to state whether the participants of this study are or are not within trends for a general population of the same age group. It does provide however an interpretation tool that could be used in future studies.

5. Conclusion

The present study shows that the prevalence of vitamin D insufficiency or deficiency is not greater in cALL survivors compared to what is reported for the general Canadian population despite low vitamin D food and supplement intakes. This is somewhat surprising as one may surmise that such patients would tend to be more alert to take supplements and worried of their nutrition. Furthermore, there seem to be no overt imbalance in the gender- and age-adjusted serum bone turnover marker concentrations. Our data indicate that regular nutritional evaluation should be part of the patients' follow-up panel.

Authors' contribution

Emile Levy, Caroline Laverdière, Nathalie Alos, Daniel Sinnett, Maja Krajinovic, Simon Drouin and Valérie Marcil conceptualized and initiated the study; Nathalie Alos and Caroline Laverdière recruited the cALL survivors and provided the clinical evaluations; Valérie Marcil and Sofia Morel collected all the nutritional data and contributed to their analyses; Michel Boisvert and Marc-André Lecours performed the analysis of vitamin D metabolites; Frank Rauch supervised the serum bone turnover biomarker analyses and provided clinical interpretation; Josée Dubois contributed by supervising and analyzing the DXA scans for clinical purposes; Mariia Samoilenko and Geneviève Lefebvre performed the statistical analyses; Carine Nyalendo supervised the biochemical analyses and provided the reference values; Etienne Cavalier provided the reference values for the bone turnover biomarkers; Edgard Delvin and Emile Levy supervised the analysis of the vitamin D metabolites, whereas Edgard Delvin analyzed the data and wrote the article. All authors revised and accepted the final version of the manuscript.

Conflicts of interest

The authors declare “Conflicts of interest: none”.

Acknowledgements

We wish to thank the cALL survivors that generously gave of their time to be part of the PETALE study. This work was supported

by the Institute of Cancer Research (ICR) of the Canadian Institutes of Health Research (CIHR), in collaboration with C17 Council, Canadian Cancer Society (CCS), Cancer Research Society (CRS), Garron Family Cancer Centre at the Hospital for Sick Children, Ontario Institute for Cancer Research (OICR), Pediatric Oncology Group of Ontario (POGO) (grant number: TCF 118694) and the J.A.DeSève Research Chair in Nutrition.

References

[1] Riedt T, Ebinger M, Salih HR, Tomiuk J, Handgretinger R, Kanz L, et al. Aberrant expression of the homeobox gene CDX2 in pediatric acute lymphoblastic leukemia. Blood 2009;113(17):4049–51.
[2] Viana SS, de Lima LM, do Nascimento JB, Cardoso CA, Rosário AC, deQ. Mendonça C, et al. Secular trends and predictors of mortality in acute lymphoblastic leukemia for children of low socioeconomic level in Northeast Brazil. Leuk Res 2015;39(10):1060–5.
[3] Hunger SP, Lu X, Devidas M, Camitta BM, Gaynon PS, Winick NJ, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol Offic J Am Soc Clin Oncol 2012;30(14):1663–9.
[4] Zhang FF, Parsons SK. Obesity in childhood cancer survivors: call for early weight management. Adv Nutr (Bethesda, Md) 2015;6(5):611–9.
[5] Chow EJ, Simmons JH, Roth CL, Baker KS, Hoffmeister PA, Sanders JE, et al. Increased cardiometabolic traits in pediatric survivors of acute lymphoblastic leukemia treated with total body irradiation. Biol Blood Marrow Transplant 2010;16(12):1674–81.
[6] Barnea D, Raghunathan N, Friedman DN, Tonorezos ES. Obesity and metabolic disease after childhood cancer. Oncology (Williston Park) 2015;29(11):849–55.
[7] Arikoski P, Komulainen J, Riikonen P, Jurvelin JS, Voutilainen R, Kroger H. Reduced bone density at completion of chemotherapy for a malignancy. Arch Dis Child 1999;80(2):143–8.
[8] Salim H, Ariawati K, Suryawan WB, Arimbawa M. Osteoporosis resulting from acute lymphoblastic leukemia in a 7-year-old boy: a case report. J Med Case Rep 2014;8:168.
[9] Rogalsky RJ, Black GB, Reed MH. Orthopaedic manifestations of leukemia in children. J Bone Jt Surg Am Vol 1986;68(4):494–501.
[10] Halton JM, Atkinson SA, Fraher L, Webber CE, Cockshott WP, Tam C, et al. Mineral homeostasis and bone mass at diagnosis in children with acute lymphoblastic leukemia. J Pediatr 1995;126(4):557–64.
[11] Boot AM, van den Heuvel-Eibrink MM, Hählen K, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children with acute lymphoblastic leukaemia. Eur J Canc 1999;35(12):1693–7.
[12] Halton J, Gaboury I, Grant R, Alos N, Cummings EA, Matzinger M, et al. Advanced vertebral fracture among newly diagnosed children with acute lymphoblastic leukemia: results of the Canadian Steroid-Associated Osteoporosis in the Pediatric Population (STOPP) research program. J Bone Miner Res Offic J Am Soc Bone Miner Res 2009;24(7):1326–34.
[13] Alikasifoglu A, Yetgin S, Cetin M, Tuncer M, Gumruk F, Gurgey A, et al. Bone mineral density and serum bone turnover markers in survivors of childhood acute lymphoblastic leukemia: comparison of megadose methylprednisolone and conventional-dose prednisolone treatments. Am J Hematol 2005;80(2):113–8.
[14] Gunes AM, Can E, Saglam H, Ilcol YO, Baytan B. Assessment of bone mineral density and risk factors in children completing treatment for acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2010;32(3):e102–7.
[15] Gurney JG, Kaste SC, Liu W, Srivastava DK, Chemaitilly W, Ness KK, et al. Bone mineral density among long-term survivors of childhood acute lymphoblastic leukemia: results from the St. Jude Lifetime Cohort Study. Pediatr Blood Cancer 2014;61(7):1270–6.
[16] Pagano-Therrien J, Santacroce SJ. Bone mineral density decrements and children diagnosed with cancer. J Pediatr Oncol Nurs Offic J Assoc Pediatr Oncol Nurses 2005;22(6):328–38.
[17] Kaste SC, Karimova EJ, Neel MD. Osteonecrosis in children after therapy for malignancy. AJR Am J Roentgenol 2011;196(5):1011–8.
[18] Prentice A, Schoenmakers I, Laskey MA, de Bono S, Ginty F, Goldberg GR. Nutrition and bone growth and development. Proc Nutr Soc 2006;65(4):348–60.
[19] Berger C, Goltzman D, Langsetmo L, Joseph L, Jackson S, Kreiger N, et al. Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. J Bone Miner Res Offic J Am Soc Bone Miner Res 2010;25(9):1948–57.
[20] Anderson PH, Atkins GJ, Turner AG, Kogawa M, Findlay DM, Morris HA. Vitamin D metabolism within bone cells: effects on bone structure and strength. Mol Cell Endocrinol 2011;347(1–2):42–7.
[21] Gunton JE, Girgis CM, Baldock PA, Lips P. Bone muscle interactions and vitamin D. Bone 2015;80:89–94.
[22] Anderson PH, Lam NN, Turner AG, Davey RA, Kogawa M, Atkins GJ, et al. The pleiotropic effects of vitamin D in bone. J Steroid Biochem Mol Biol 2013;136:190–4.
[23] Atkins GJ, Kostakis P, Pan B, Farrugia A, Gronthos S, Evdokiou A, et al. RANKL expression is related to the differentiation state of human osteoblasts. J Bone Miner Res Offic J Am Soc Bone Miner Res 2003;18(6):1088–98.
[24] Henry HL. Regulation of vitamin D metabolism. Best Pract Res Clin Endocrinol Metabol 2011;25(4):531–41.
[25] Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino ACW, et al. Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1α,25-dihydroxyvitamin D3. Bone 2007;40(6):1517–28.
[26] Kogawa M, Anderson PH, Findlay DM, Morris HA, Atkins GJ. The metabolism of 25-(OH)vitamin D3 by osteoclasts and their precursors regulates the differentiation of osteoclasts. J Steroid Biochem Mol Biol 2010;121(1–2):277–80.
[27] Need AG, Horowitz M, Morris HA, Nordin BC. Vitamin D status: effects on parathyroid hormone and 1, 25-dihydroxyvitamin D in postmenopausal women. Am J Clin Nutr 2000;71(6):1577–81.
[28] Haarburger D, Hoffman M, Erasmus RT, Pillay TS. Relationship between vitamin D, calcium and parathyroid hormone in Cape Town. J Clin Pathol 2009;62(6):567–9.
[29] Fillee C, Keller T, Mourad M, Brinkmann T, Ketelslegers JM. Impact of vitamin D-related serum PTH reference values on the diagnosis of mild primary hyperparathyroidism, using bivariate calcium/PTH reference regions. Clin Endocrinol 2012;76(6):785–9.
[30] Gedikoglu G, Altinoz MA. Differentiation-inducing liposoluble vitamin deficiency may explain frequent secondary solid tumors after hematopoietic stem cell transplantation: minireview. Neoplasma 2008;55(1):1–9.
[31] Stein EM, Shane E. Chapter 68-vitamin D and organ transplantation A2 – Adams, David Feldman J. Wesley Pike John S. Vitamin D. 3rd ed. San Diego: Academic Press; 2011. p. 1291–8.
[32] Simmons JH, Chow EJ, Koehler E, Esbenshade A, Smith LA, Sanders J, et al. Significant 25-hydroxyvitamin D deficiency in child and adolescent survivors of acute lymphoblastic leukemia: treatment with chemotherapy compared with allogeneic stem cell transplant. Pediatr Blood Cancer 2011;56(7):1114–9.
[33] Modan-Moses D, Pinhas-Hamiel O, Munitz-Shenkar D, Temam V, Kanety H, Toren A. Vitamin D status in pediatric patients with a history of malignancy. Pediatr Res 2012;72(6):620–4.
[34] Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, et al. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab 2016;101(2):394–415.
[35] Krajinovic M, Labuda D, Richer C, Karimi S, Sinnett D. Susceptibility to childhood acute lymphoblastic leukemia: influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 genetic polymorphisms. Blood 1999;93(5):1496–501.
[36] Sinnett D, Krajinovic M, Labuda D. Genetic susceptibility to childhood acute lymphoblastic leukemia. Leuk Lymphoma 2000;38(5–6):447–62.
[37] Krajinovic M, Elbared J, Drouin S, Bertout L, Rezgui A, Ansari M, et al. Polymorphisms of ABCC5 and NOS3 genes influence doxorubicin cardiotoxicity in survivors of childhood acute lymphoblastic leukemia. Pharmacogenom J 2016;16:530–5.
[38] Marcoux S, Drouin S, Laverdiere C, Alos N, Andelfinger GU, Bertout L, et al. The PETALE study: late adverse effects and biomarkers in childhood acute lymphoblastic leukemia survivors. Pediatr Blood Cancer 2017 Jun;64(6). https://doi.org/10.1002/pbc.26361. Epub 2016 Dec 4.
[39] Vrooman LM, Neuberg DS, Stevenson KE, Asselin BL, Athale UH, Clavell L, et al. The low incidence of secondary acute myelogenous leukaemia in children and adolescents treated with dexrazoxane for acute lymphoblastic leukaemia: a report from the Dana-Farber Cancer Institute ALL Consortium. Eur J Cancer 2011;47(9):1373–9.
[40] Lambert M, Delvin EE, Levy E, O'Loughlin J, Paradis G, Barnett T, et al. Prevalence of cardiometabolic risk factors by weight status in a population-based sample of Quebec children and adolescents. Can J Cardiol 2008;24(7):575–83.
[41] Statement PP. The use of growth charts for assessing and monitoring growth in Canadian infants and children. Can J Diet Pract Res Publ Diet Can Revue canadienne de la pratique et de la recherche en dietetique : une publication des Dietetistes du Canada 2004;65(1):22–32.
[42] Alberti KG, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. Lancet (London, England) 2005;366(9491):1059–62.
[43] Third Report of the National Cholesterol Education Program (NCEP). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation 2002;106(25):3143–421.
[44] Katzmarzyk PT. Waist circumference percentiles for Canadian youth 11-18y of age. Eur J Clin Nutr 2004;58(7):1011–5.
[45] World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation. 2008. http://www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/.
[46] Tholen DW, Anders K, Kenndy JW, Krouwer JS, Meier K. Evaluation of precision performance of quantitative measurement methods, approved guidelines. 2nd ed., vol. 24. CLSI; 2004. p. 1–39.
[47] Kaufmann M, Gallagher JC, Peacock M, Schlingmann KP, Konrad M, DeLuca HF, et al. Clinical utility of simultaneous quantitation of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD. J Clin Endocrinol Metab 2014;99(7):2567–74.
[48] Delvin E, Boisvert M, Lecours MA, Théorêt Y, Kaufmann M, Jones G, et al. Supporting data for the measurement of serum vitamin D metabolites in childhood acute lymphoblastic leukemia survivors. Data in Brief. 2018.

[49] Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011. Report No.: 978-0-309-16394-1.
[50] Weiler HA, Leslie WD, Krahn J, Steiman PW, Metge CJ. Canadian Aboriginal women have a higher prevalence of vitamin D deficiency than non-Aboriginal women despite similar dietary vitamin D intakes. J Nutr 2007;137(2):461–5.
[51] Anderson LN, Cotterchio M, Vieth R, Knight JA. Vitamin D and calcium intakes and breast cancer risk in pre- and postmenopausal women. Am J Clin Nutr 2010;91(6):1699–707.
[52] Zhou W, Langsetmo L, Berger C, Poliquin S, Kreiger N, Barr SI, et al. Longitudinal changes in calcium and vitamin D intakes and relationship to bone mineral density in a prospective population-based study: the Canadian Multicentre Osteoporosis Study (CaMos). J Musculoskelet Neuronal Interact 2013;13(4):470–9.
[53] Mark S, Gray-Donald K, Delvin EE, O'Loughlin J, Paradis G, Levy E, et al. Low vitamin D status in a representative sample of youth from Quebec, Canada. Clin Chem 2008;54(8):1283–9.
[54] Langlois K, Greene-Finestone L, Little J, Hidiroglou N, Whiting S. Vitamin D status of canadians as measured in the 2007 to 2009 Canadian health measures survey. Health Rep 2010;21(1):47–55.
[55] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metabol 2011;96(7):1911–30.
[56] Glendenning P, Inderjeeth CA. Screening for vitamin D deficiency: defining vitamin D deficiency, target thresholds of treatment and estimating the benefits of treatment. Pathology 2012;44(2):160–5.
[57] Crews BO, Moore J, Dietzen DJ. Circulating intact parathyroid hormone is suppressed at 25-hydroxyvitamin D concentrations >25 nmol/L in children. J Pediatr Endocrinol Metab 2014;27(7–8):657–60.
[58] Souberbielle JC, Massart C, Brailly-Tabard S, Cormier C, Cavalier E, Delanaye P, et al. Serum PTH reference values established by an automated third-generation assay in vitamin D-replete subjects with normal renal function: consequences of diagnosing primary hyperparathyroidism and the classification of dialysis patients. Eur J Endocrinol 2016;174(3):315–23.
[59] Ding C, Parameswaran V, Blizzard L, Burgess J, Jones G. Not a simple fat-soluble vitamin: changes in serum 25-(OH)D levels are predicted by adiposity and adipocytokines in older adults. J Intern Med 2010;268(5):501–10.
[60] Jorde R, Sneve M, Emaus N, Figenschau Y, Grimnes G. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromso study. Eur J Nutr 2010;49(7):401–7.
[61] Mai XM, Chen Y, Camargo Jr CA, Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study. Am J Epidemiol 2012;175(10):1029–36.
[62] Hannemann A, Thuesen BH, Friedrich N, Volzke H, Steveling A, Ittermann T, et al. Adiposity measures and vitamin D concentrations in Northeast Germany and Denmark. Nutr Metabol 2015;12:24.
[63] Michelsen J, Wallaschofski H, Friedrich N, Spielhagen C, Rettig R, Ittermann T, et al. Reference intervals for serum concentrations of three bone turnover markers for men and women. Bone 2013;57(2):399–404.
[64] Bayer M. Reference values of osteocalcin and procollagen type I N-propeptide plasma levels in a healthy Central European population aged 0-18 years. Osteoporos Intl J Establ Res Coop Between Eur Found Osteoporos Natl Osteoporos Found USA 2014;25(2):729–36.
[65] Hannemann A, Wallaschofski H. Reference intervals for serum concentrations of three bone turnover markers for men and women. Bone 2016;93:216.
[66] Callegari ET, Gorelik A, Garland SM, Chiang CY, Wark JD. Bone turnover marker reference intervals in young females. Ann Clin Biochem 2017 Jul;54(4):438–47. https://doi.org/10.1177/0004563216665123. Epub 2016 Aug 5.
[67] Madar AA, Knutsen KV, Stene LC, Brekke M, Lagerløv P, Meyer HE, et al. Effect of vitamin D3-supplementation on bone markers (serum P1NP and CTX): a randomized, double blinded, placebo controlled trial among healthy immigrants living in Norway. Bone Reports 2015;2:82–8.
[68] Ginty F, Cavadini C, Michaud PA, Burckhardt P, Baumgartner M, Mishra GD, et al. Effects of usual nutrient intake and vitamin D status on markers of bone turnover in Swiss adolescents. Eur J Clin Nutr 2004;58(9):1257–65.
[69] Thiering E, Bruske I, Kratzsch J, Hofbauer LC, Berdel D, von Berg A, et al. Associations between serum 25-hydroxyvitamin D and bone turnover markers in a population based sample of German children. Sci Rep 2015;5:18138.
[70] Tonks KT, White CP, Center JR, Samocha-Bonet D, Greenfield JR. Bone turnover is suppressed in insulin resistance, independent of adiposity. J Clin Endocrinol Metab 2017;102(4):1112–21.