



Original article

Bone status and adipokine levels in children on vegetarian and omnivorous diets



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SUMMARY

Background & aims: Measurements of bone mineral density (BMD) reflect bone status but not the dynamics of bone turnover. Biochemical markers, which show global skeletal activity, were validated for the assessment of bone formation and resorption processes. Adipokines also play a significant role in the regulation of bone metabolism.

Objective: To assess body composition, bone mineral density, bone turnover markers and adipokine levels in relation to vegetarian and omnivorous diets.

Methods: The study included 53 vegetarian and 53 omnivorous prepubertal healthy children matched for age and sex (median age 7.0 years). Body composition and BMD were assessed by dual-energy X-ray absorptiometry. 25-hydroxyvitamin D and parathormone levels were measured by chemiluminescence method. Serum carboxy-terminal propeptide of type I collagen (CICP), total osteocalcin (OC) and its forms carboxylated (c-OC) and undercarboxylated (uc-OC), C-terminal cross-linking telopeptide of collagen type I (CTX), leptin and adiponectin levels were determined using immunoenzymatic assays.

Results: Both groups of children were comparable in terms of body composition, except for the percentage of fat mass, which was lower (19.24 vs. 21.77%, $p = 0.018$) in vegetarians. Mean values of total BMD z-score and lumbar spine BMD z-score were lower (-0.583 vs. -0.194 , $p = 0.009$ and -0.877 vs. -0.496 , $p = 0.019$, respectively) in vegetarians compared with omnivores. Serum leptin level was about 2-fold lower (1.39 vs. 2.94 ng/mL, $p < 0.001$) in vegetarians, however, adiponectin concentration was similar in both groups. Vegetarians had similar concentration of 25-hydroxyvitamin D, but higher parathormone (40.8 vs. 32.1 pg/mL, $p = 0.015$) and CTX (1.94 vs. 1.76 ng/mL, $p = 0.077$) levels than omnivores. Total osteocalcin and CICP concentrations were comparable in both groups, however, c-OC/uc-OC ratio was higher (1.43 vs. 1.04 ng/mL, $p < 0.05$) in vegetarians. We found positive correlation between c-OC and nutritional parameters adjusted for total energy intake (plant protein, phosphorus, magnesium and fiber intakes) in vegetarian children.

Conclusions: Prepubertal children on a vegetarian diet had significantly lower total and lumbar spine BMD z-scores, but absolute values of bone mineral density did not differ. BMD z-scores did not correlate with bone metabolism markers and nutritional variables, but were positively associated with anthropometric parameters. Lower leptin levels in vegetarian children reflect lower body fat. Longitudinal studies are necessary to evaluate the impact of the observed association on bone health at adulthood.

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1. Introduction

Vegetarian diets attract more and more attention among families due to the reports about health benefits confirmed by several clinical studies. According to the statement of the American Dietetic Association, well-planned vegetarian diet, can be adequate for all

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stages of the life cycle, including childhood [1–4]. It is important to note that disturbances in body composition, especially in fat mass and its distribution are associated with alterations in the synthesis of adipokines by adipose tissue. Adipokines, such as leptin and adiponectin are of special interest due to their relationship with body weight and opposing biologic functions, including the possible role in the regulation of bone metabolism [5–7]. Bone status may be assessed by measurements of bone mineral density (BMD) and through noninvasive determination of biochemical bone turnover markers, which show global skeletal activity and can detect subtle alterations in the skeleton over short periods of time. Among bone markers, there are indicators of bone formation and bone resorption processes. Markers of bone formation are either generated from newly synthesized collagen such as carboxy-terminal propeptide of type I collagen (CICP) or osteoblast-related protein such as osteocalcin (OC). Osteocalcin which reflects osteoblast activity may exist in two forms: fully carboxylated osteocalcin (c-OC) and undercarboxylated (uc-OC) osteocalcin, in which only 0–2 residues are carboxylated. Carboxylated osteocalcin, through binding calcium and consequently hydroxyapatite, plays an active regulatory role in bone formation and mineralization, however, undercarboxylated OC has an effect on other metabolic processes, such as glucose homeostasis [8–10]. The majority of bone resorption markers are products derived from degradation of collagen molecule. C-terminal telopeptide of collagen type I (CTX) is a cross-linked peptide, which is liberated into circulation and may be determined in serum. Elevated level of CTX is correlated with increase of bone resorption [11]. Serum levels of bone turnover markers are not stable throughout life being higher in infants, children and adolescents than in adults [12,13]. Children have increased concentrations of bone markers due to their skeletal growth velocity and high rate of bone turnover. The widespread applicability of bone metabolism markers is limited by the availability of normative data in pediatric population [14,15].

Since nutrition is one of the determinants of bone health, the sufficiency of mineral elements (calcium, phosphorus, magnesium) and vitamins (vitamin D, vitamin K) are essential for organic bone matrix synthesis. The insufficiency of these nutrients is closely related to bone metabolism, with increased bone turnover, decreased bone mineral acquisition and increased risk of osteoporosis [16–18]. Some of these nutrients in vegetarian diets need specific attentions, especially in children and adolescents [19–21].

The purpose of this study was a) to assess body composition and adipokine levels, b) to evaluate bone status through assessing bone metabolism markers and bone mineral parameters, c) to investigate relationships between anthropometric, biochemical and nutrient parameters in prepubertal children on lacto-ovo-vegetarian and omnivorous diets.

2. Material and methods

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Mother and Child (decision number 13/2012). The parents of studied children gave written informed consent before participation in the study.

At present, there are about 150 vegetarian children (lacto-ovo-vegetarians, lacto-vegetarians, ovo-vegetarians, vegans) on medical and nutritional care at the Institute of Mother and Child. We included in the study the maximum possible number of vegetarian children who followed inclusion criteria (being on a lacto-ovo-vegetarian diet from birth, in the prepubertal period, generally healthy without development and nutrition disorders, BMI z-score between –1 and +1) and had densitometry as well as biochemical examination done simultaneously. Healthy children (consuming

meat, fish, and poultry) matched for age and sex which were recruited from consecutive patients attending Institute of Mother and Child who follow inclusion criteria (except of being vegetarians) were included to the study as omnivorous group. The exclusion criteria were: not in the prepubertal period, history of low birth weight, gastrointestinal diseases accompanied by malabsorption, history of chronic renal failure, chronic infection, chronic drug consumption or drug use that negatively affected bone metabolism. These rigorous selection criteria were applied in order to minimize interference from factors that could affect normal bone metabolism.

Our study group consisted of 53 prepubertal children (age range 5–10 years) following a lacto-ovo-vegetarian diet, who did not consume meat, fish, poultry, but consumed eggs, milk, and dairy products. The control group included 53 healthy prepubertal children matched for age and sex following a traditional omnivorous diet. The children were recruited between February 2014 and July 2016.

Pubertal stage was assessed according to the Tanner scale by pediatrician. All studied children were at Tanner I stage. A similar number of children on a vegetarian diet and omnivores were examined in the autumn–winter and spring–summer periods. About 80% of vegetarian and 80% of omnivorous children got vitamin D supplements (mean value 800 IU/day).

Based on the data obtained from children and parents, the level of physical activity was similar in both studied groups. According to World Health Organization guidelines (regarding physical activity), children and adolescents aged 5–17 years should accumulate 60 min of moderate-to-vigorous physical activity (MVPA) each day. Evidence also suggests that they should engage in vigorous physical activity (VPA) at least 3 days a week. The studied children (vegetarians and omnivores) were attaining these recommendations and accumulated about 60–90 min daily of MVPA and approximately 30 min per day of VPA (it was activities after school twice a week for 1 or 2 h).

Methods used for measuring dietary intake in studied children were described in detail previously [22]. Data from three consecutive days (two weekdays and one weekend day) were analyzed by nutritional software program Dieta5® in order to evaluate daily total energy intake, the percentage of energy from protein, carbohydrates and fat, as well as dietary minerals and vitamins (calcium, phosphorus, magnesium, vitamin D) intakes in the children's diets [23]. Complete dietary data were available for a smaller subgroup of study participants. Because the analysis in our work was done in pairs (as Δ variables between vegetarians and omnivores value) only 25 pairs were included in dietary analyses. The obtained results were compared to the current Polish recommendations for energy and nutrient intakes. The age- and sex specific percentage of EER (Estimated Energy Requirement) for total energy intake, DRI (Dietary Reference Intake) for protein, phosphorus, and magnesium intakes, EAR (Estimated Average Requirement) for calcium intake and AI (Adequate Intake) for fiber and vitamin D intakes were calculated using reference values of daily energy intake for children according to Jarosz et al. [24]. Since our studied children are between 5 and 11 years old they fall into the three groups mentioned in the above recommendations (group: 4–6 years, 7–9 years and 10–12 years). The data for each child was compared to the recommendations for appropriate age and gender.

Measurements of body height and weight were performed in all children and body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). Body composition (percentage of fat, fat mass, lean mass) and bone mineral density in the total body less head (TBLH-BMD) and in the lumbar spine L2–L4 (BMDL2–L4) were measured by dual-energy x-ray absorptiometry (DXA) using Lunar Prodigy densitometer (General Electric

Healthcare, Madison, WI). We decided on using TBLH-BMD according to Official Positions of International Society for Clinical Densitometry 2013 [25]. BMD z-scores were obtained using pediatric reference population database enCORE software version 9.30.044.

Venous blood was taken after an overnight fast in the morning (8–10 a.m.) for biochemical analyses. Serum samples were collected after centrifugation (1000×g for 10 min at 4 °C) and stored frozen (–20 °C) until analysis (no longer than 2 months). Levels of adipokines (leptin and adiponectin) were determined by kits from DRG Diagnostics (Marburg, Germany). Intra- and inter-assay variations were 5.95% and 8.66% for leptin and 7.4% and 8.4% for adiponectin, respectively. Serum levels of 25-hydroxyvitamin D and parathyroid hormone (PTH) were determined by electrochemiluminescence method (ECLIA) with kits from DiaSorin Inc. (Stillwater, USA). Concentrations of bone metabolism markers were determined using commercial immuno-enzymatic assay (ELISA). Serum OC and CTX concentrations were measured with kits from IDS (Baldon, UK). The intra- and inter-assay coefficients of variation (CVs) were 1.3–2.2% and 2.7–5.1% for OC and 1.7–3.0% and 2.5–10.9% for CTX, respectively. Levels of c-OC and uc-OC forms of osteocalcin in serum were determined using kits from Takara Bio Inc. (Shiga, Japan), in which intra- and inter-assay CVs were 3.0–4.8% and 0.7–2.4% for c-OC and 4.4–6.6% and 5.6–9.8% for uc-OC, respectively. Serum C1CP concentration was determined with kits from Quidel (San Diego, USA) with intra-assay CVs from 5.5% to 6.8% and inter-assay CVs from 5.0% to 7.2%.

All statistical analyses were performed using IBM-SPSS software version 23.0 (SPSS INC., Chicago, IL., USA). The normality of variables was tested by Shapiro–Wilk test. Normally distributed data were presented as means and standard deviation (SD) and non-normally distributed variables as median and interquartile range (25th–75th percentiles). Anthropometric characteristics, dietary intake and biochemical parameters of vegetarian and omnivorous children were compared by means of paired t-Students test or non-parametric Wilcoxon test. The magnitude of differences between studied parameters in each pair of matched subjects was expressed as Δ variables, calculated by subtracting values of vegetarians minus values of omnivores. To evaluate the relationships between Δ variables concerning bone mineral density, nutrient intakes, body composition, and biochemical markers correlation analysis was applied. To adjust nutrients intake for energy intake in the vegetarian group, residuals from the regression model with total caloric intake as the independent variable and log-transformed absolute intakes of a given nutrient as the dependent variable were used in the analysis of associations with serum markers of bone metabolism (residual method). We also computed relationships between unadjusted nutrient intakes and biochemical bone markers. For bivariate analysis of associations Spearman correlation was used. The differences were regarded as statistically significant at $p < 0.05$.

3. Results

The data on children's characteristic and anthropometry are presented in Table 1. We did not observe significant differences in studied parameters between subgroups of girls and boys. That is why we analyzed the obtained data as only one group of vegetarian children and one group of omnivorous children. Generally, the growth and development of all children was normal and there were no significant differences in weight, height, BMI and BMI z-score between vegetarians and omnivores. However, vegetarian subjects had significantly lower (19.24 vs. 21.77, $p = 0.018$) percentage of fat mass and ratio of fat to lean mass (0.24 vs. 0.29, $p = 0.013$). The mean values of TBLH-BMD z-score and lumbar spine BMD L2–L4 z-

score were significantly lower (–0.583 vs. –0.194, $p = 0.009$ and –0.877 vs. –0.496, $p = 0.019$, respectively) in children on vegetarian than in those on a traditional diet.

Regarding adipokines, we noticed above 2-fold lower median value of serum leptin (1.39 vs. 2.94 ng/mL, $p < 0.001$) and similar adiponectin levels in vegetarian children compared with their omnivorous peers (Table 2). Hence, the ratio of leptin/adiponectin was significantly (0.14 vs. 0.31, $p < 0.001$) lower in children on a vegetarian diet.

Among bone metabolism markers, we observed slightly higher (1.942 vs. 1.763 ng/mL, $p = 0.077$) concentration of bone resorption marker – CTX in the group of vegetarians than in omnivores and comparable concentrations of bone formation markers – C1CP and osteocalcin. Hence, OC/CTX ratio was significantly lower (43.31 vs. 49.33, $p = 0.039$) in vegetarians compared with non-vegetarians. Serum levels of carboxylated and undercarboxylated form of osteocalcin did not differ significantly in both studied groups, however, we found that c-OC level tended to be higher (33.3 vs. 29.3 ng/mL, $p = 0.077$) in vegetarians. Hence, vegetarians had a significantly higher (1.43 vs. 1.04, $p = 0.010$) ratio of c-OC to uc-OC. We also found out, significantly (40.8 vs. 32.1 pg/mL, $p = 0.015$) higher serum levels of PTH in vegetarians compared with omnivores and similar concentrations of 25-hydroxyvitamin D. Despite similar mean levels of serum 25 hydroxyvitamin D in both groups of children, 36% of vegetarian children had vitamin D deficiencies (<20 ng/mL), 38% had insufficient (20–30 ng/mL) and 26% had sufficient (>30 ng/mL) level of this vitamin. In omnivorous group, 30% of children had deficiencies in vitamin D level, 41% – insufficient level and 29% – optimal level.

Based on a small subgroup of study participants (25 vegetarians and 25 omnivores), daily energy and macronutrient intake was within the recommended data, whereas the proportions of proteins and carbohydrates in vegetarian and omnivorous diets were different (Table 3). Vegetarians compared with omnivores, had a significantly lower protein intake (37.7 vs. 54.8 g/day, $p = 0.002$) and the percentage of energy from protein (11.0 vs. 14.1%, $p = 0.001$) but higher percentage of energy from carbohydrates (58.2 vs. 52.0%, $p = 0.001$). As expected, in vegetarian diet the intake of animal protein was lower (11.0 vs. 35.5 g/day, $p < 0.001$) and plant protein higher (24.7 vs. 17.9 g/day, $p = 0.018$) compared with omnivorous diet. In both studied groups, dietary intakes of calcium, phosphorus and vitamin D were similar, but intakes of fiber and magnesium were higher ($p = 0.015$ and $p = 0.054$, respectively) in vegetarian diet. We assessed the amount of protein and calcium from dairy in both groups of children and found that median value of protein intake from these products was slightly lower (but without statistical difference) in vegetarians: 11.0 g/day (3.4–16.0 g/day) than in omnivores: 12.2 g/day (6.5–15.8 g/day) ($p = 0.397$). Also, median value of the amount of calcium from dairy was lower in vegetarians compared with omnivorous children (309 mg/day (107–451 mg/day) vs. 369 mg/day (189–514 mg/day), $p = 0.239$).

Analyzing the age- and sex specific dietary reference intake percentage in vegetarians, about 85% of children followed recommended intake for energy and all children for protein, phosphorus and magnesium intake. However, only about 54% of vegetarians and 62% of omnivores followed recommended daily intake for calcium and 40% of vegetarians and 42% of non vegetarians for vitamin D.

We found significant positive correlations between Δ TBLH-BMD z-score and anthropometric variables: Δ body mass, Δ height, Δ BMI z-score, Δ fat, Δ lean, Δ ratio of fat/lean and Δ BMDL2–L4 z-score (Table 4). Notably, there were no associations between Δ TBLH-BMD z-score and serum concentrations of adipokines (leptin and adiponectin) as well as biochemical bone markers, except for a negative correlation with Δ C1CP ($r = -0.394$, $p = 0.038$). Moreover,

Table 1
Clinical and anthropometric data in vegetarian and omnivorous children.

	Vegetarian children (n = 53)	Omnivorous children (n = 53)	Δ	p
Age (years) ^b	7.0 (5.3–9.0)	7.0 (5.4–8.9)	0.0 (–0.3–0.0)	0.314
Weight (kg) ^b	21.0 (18.0–28.8)	21.4 (18.2–27.0)	0.6 (–2.0–3.0)	0.771
Height (cm) ^a	122.2 ± 13.2	123.2 ± 13.3	–0.9 ± 8.0	0.414
Height z-score ^a	–0.45 ± 1.06	–0.29 ± 1.22	–0.17 ± 1.45	0.425
BMI (kg/m ²) ^b	15.4 (14.5–16.1)	14.9 (14.0–16.0)	0.5 (–0.7–1.6)	0.270
BMI z-score ^b	–0.48 (–0.85–0.08)	–0.57 (–1.08–0.05)	0.24 (–0.39–0.87)	0.295
Fat (%) ^a	19.24 ± 5.33	21.77 ± 7.60	–2.53 ± 7.46	0.018
Fat (kg) ^b	3.63 (2.81–5.43)	3.79 (3.12–5.69)	–0.15 (–1.76–1.11)	0.244
Lean (kg) ^a	17.51 ± 4.33	17.06 ± 4.59	0.45 ± 4.16	0.437
Fat/lean ^a	0.24 ± 0.08	0.29 ± 0.13	–0.04 ± 0.12	0.013
TBLH-BMD (g/cm ²) ^a	0.628 ± 0.077	0.640 ± 0.090	–0.012 ± 0.085	0.310
TBLH-BMD z-score ^a	–0.583 ± 0.718	–0.194 ± 0.642	–0.388 ± 1.024	0.009
BMDL2-L4 (g/cm ²) ^a	0.621 ± 0.089	0.649 ± 0.096	–0.028 ± 0.097	0.046
BMDL2-L4 z-score ^a	–0.877 ± 0.858	–0.496 ± 0.791	–0.381 ± 1.132	0.019

Data are presented as ^amean values ± standard deviation (SD) and t-Student paired test was used for normally distributed Δ variables or as ^bmedian values and interquartile ranges and Wilcoxon test was used for non-normally distributed Δ variables; Δ – the differences between value of vegetarians minus value of omnivores; BMI – body mass index, TBLH – total bone mineral density less head, BMDL2-L4 – lumbar spine L2–L4 bone mineral density.

Table 2
Serum concentrations of adipokines and bone metabolism markers in vegetarian and omnivorous children.

	Vegetarians (n = 53)	Omnivores (n = 53)	Δ	p
Leptin (ng/mL) ^b	1.39 (0.89–2.22)	2.94 (1.60–3.95)	–1.16 (–2.54–0.20)	<0.001
Adiponectin (ng/mL) ^a	8.92 ± 2.32	8.55 ± 3.09	0.37 ± 4.39	0.543
Leptin/adiponectin ratio ^b	0.14 (0.10–0.24)	0.31 (0.20–0.50)	–0.17 (–0.32–0.00)	<0.001
CTX (ng/mL) ^a	1.942 ± 0.561	1.763 ± 0.577	0.179 ± 0.720	0.077
OC (ng/mL) ^a	80.5 ± 28.7	81.4 ± 27.3	–0.9 ± 41.8	0.876
c-OC (ng/mL) ^a	33.3 ± 11.7	29.3 ± 9.1	4.0 ± 15.5	0.077
uc-OC (ng/mL) ^b	26.0 (17.2–39.2)	30.0 (22.4–38.1)	–5.3 (–17.3–15.5)	0.249
CICP (ng/mL) ^a	319.0 ± 92.8	325.8 ± 113.4	–6.8 ± 159.6	0.819
OC/CTX ratio ^a	43.31 ± 15.66	49.33 ± 18.40	–6.01 ± 20.72	0.039
c-OC/uc-OC ratio ^a	1.43 ± 0.81	1.04 ± 0.52	0.39 ± 1.01	0.010
c-OC/OC ratio ^a	0.45 ± 0.16	0.39 ± 0.13	0.06 ± 0.20	0.048
uc-OC/OC ratio	0.36 ± 0.12	0.41 ± 0.11	–0.05 ± 0.18	0.079
PTH (pg/mL) ^b	40.8 (29.5–57.2)	32.1 (23.1–42.5)	10.4 (–5.9–33.4)	0.015
25 hydroxyvitamin D (ng/mL) ^a	24.2 ± 10.4	25.5 ± 9.0	–1.3 ± 12.8	0.469

Data are presented as ^amean values ± standard deviation (SD) and t-Student paired test for normally distributed Δ variables or as ^bmedian values and interquartile ranges and Wilcoxon test for non-normally distributed Δ variables; Δ – the difference between value of vegetarians minus value of omnivores; PTH – parathormone, CTX – C-terminal telopeptide of collagen type I, OC – osteocalcin, c-OC – carboxylated osteocalcin, uc-OC – undercarboxylated osteocalcin, CICP – carboxy-terminal propeptide of type I collagen.

Table 3
Daily intake of energy and nutrients in the examined children's diets¹.

Energy/Nutrients	Vegetarian children (n = 25)	Omnivorous children (n = 25)	Δ	p
Total energy (kcal) ^a	1376 ± 415	1615 ± 476	–239 ± 732	0.116
Energy from protein (%) ^a	11.0 ± 1.6	14.1 ± 3.3	–3.1 ± 4.0	0.001
Energy from fat (%) ^a	30.8 ± 4.4	33.9 ± 5.6	–3.1 ± 7.3	0.043
Energy from carbohydrates (%) ^a	58.2 ± 4.7	52.0 ± 6.1	6.2 ± 8.1	0.001
Energy (% of EER) ^a	86.1 ± 29.8	101.9 ± 33.4	–15.8 ± 47.8	0.112
Protein (g) ^a	37.7 ± 13.0	54.8 ± 16.4	–17.0 ± 24.9	0.002
Protein (% of DRI) ^a	148.8 ± 63.2	217.6 ± 84.2	–68.8 ± 113.9	0.006
Animal protein (g) ^a	11.0 ± 6.9	35.5 ± 14.9	–24.5 ± 17.5	<0.001
Plant protein (g) ^a	24.7 ± 10.6	17.9 ± 6.9	6.7 ± 13.3	0.018
Calcium (mg) ^a	460 ± 211	529 ± 154	–68 ± 313	0.285
Calcium (% of EAR) ^a	53.5 ± 22.7	62.4 ± 18.7	–8.9 ± 34.9	0.213
Phosphorus (mg) ^a	794 ± 296	884 ± 206	–90 ± 429	0.304
Phosphorus (% of DRI) ^a	132.7 ± 62.4	149.0 ± 52.8	–16.3 ± 79.0	0.312
Magnesium (mg) ^a	248 ± 116	193 ± 57	55 ± 137	0.054
Magnesium (% of DRI) ^a	172.3 ± 90.9	133.2 ± 40.5	39.0 ± 100.7	0.064
Vitamin D (μg) ^b	1.99 (0.77–4.32)	2.12 (1.13–4.25)	–0.42 (–2.13–2.63)	0.946
Vitamin D (% of AI) ^b	39.7 (15.3–86.5)	42.4 (22.6–84.9)	–8.4 (–42.6–52.6)	0.946
Fiber (g) ^a	20.4 ± 10.1	14.2 ± 5.1	6.2 ± 11.9	0.015
Fiber (% of AI) ^a	134.5 ± 69.0	92.5 ± 30.6	42.0 ± 79.8	0.015

Results are presented as ^amean values ± standard deviation (SD) and t-Student paired test was used for normally distributed Δ variables or as ^bmedian values and interquartile ranges and Wilcoxon test was used for non-normally distributed Δ variables; Δ – the difference between value of vegetarians minus value of omnivores; EER – Estimated Energy Requirement, DRI – Dietary Reference Intake, EAR – Estimated Average Requirement, AI– Adequate Intake, ¹according to Jarosz et al. [24].

Table 4
Spearman correlations between Δ TBLH-BMD z-score as well as Δ BMDL2-L4 z-score and anthropometric and biochemical parameters.

	Δ TBLH-BMD z-score		Δ BMDL2-L4 z-score	
	r	p	r	p
Anthropometric parameters				
Δ body mass	0.461	0.001	0.374	0.006
Δ body height	0.413	0.002	0.420	0.002
Δ BMI z-score	0.346	0.012	0.225	0.109
Δ fat	0.399	0.003	0.364	0.008
Δ lean	0.346	0.012	0.291	0.036
Δ fat/lean ratio	0.324	0.019	0.279	0.045
Δ BMDL2-L4 z-score	0.862	<0.001	–	–
Biochemical markers				
Δ leptin	0.185	0.195	0.114	0.424
Δ adiponectin	0.152	0.287	0.196	0.167
Δ leptin/adiponectin ratio	0.108	0.452	0.015	0.915
Δ 25 hydroxyvitamin D	0.047	0.741	0.045	0.752
Δ PTH	0.135	0.493	0.093	0.639
Δ CICP	–0.394	0.038	–0.201	0.306
Δ OC	0.078	0.584	0.191	0.176
Δ CTX	0.052	0.716	0.072	0.611
Δ c-OC	–0.023	0.877	0.017	0.907
Δ uc-OC	–0.031	0.838	0.062	0.678
Δ OC/CTX	0.036	0.799	0.146	0.300
Δ c-OC/uc-OC	0.028	0.851	0.029	0.849

BMI – body mass index, TBLH – total bone mineral density less head, BMDL2-L4 – lumbar spine L2–L4 bone mineral density, PTH – parathormone, CTX – C-terminal telopeptide of collagen type I, OC – osteocalcin, c-OC – carboxylated osteocalcin, uc-OC – undercarboxylated osteocalcin, CICP – carboxy-terminal propeptide of type I collagen, Δ – the differences between value of vegetarians minus value of omnivores.

Δ TBLH-BMD z-score did not correlate with nutritional parameters (data not shown). Similarly, Δ BMD L2–L4 z-score was correlated with anthropometric parameters, but no correlations between Δ BMD L2–L4 z-score and biochemical or nutritional variables were found. In addition, we noted that Δ leptin was related with anthropometric variables: Δ BMI z-score ($r = 0.341$, $p = 0.014$), Δ fat mass ($r = 0.487$, $p < 0.001$) and Δ fat/lean ratio ($r = 0.440$, $p = 0.001$) but not related to bone metabolism markers and nutritional parameters. Δ adiponectin was not related to anthropometric and

biochemical variables, however, was correlated with some nutritional parameters: positively with Δ % energy from carbohydrates ($r = 0.399$, $p = 0.048$) and negatively with Δ calcium ($r = -0.473$, $p = 0.017$).

In the group of vegetarian children, we observed that serum levels of total osteocalcin ($r = 0.367$, $p = 0.025$), carboxylated form of OC ($r = 0.314$, $p = 0.059$) and undercarboxylated OC ($r = 0.339$, $p = 0.040$) were positively correlated with dietary fiber (Table 5). There was no significant association between CTX and dietary nutrient intakes. After energy adjustment of intake, we found that in children following vegetarian diet, carboxylated form of osteocalcin was positively correlated not only with dietary fiber ($r = 0.488$, $p = 0.002$) but also with plant protein ($r = 0.366$, $p = 0.026$), phosphorus ($r = 0.348$, $p = 0.035$) and magnesium ($r = 0.486$, $p = 0.002$) intakes. No correlations were observed between dietary nutrients after energy adjustment of intake and CTX, total OC and uc-OC in this group of children.

4. Discussion

Nutrition in childhood is very important to ensure their normal growth and development including bone health. Some nutrients are essential for the growth of the skeleton and other are involved in the synthesis of collagen or cartilage while some of them support phosphate and calcium homeostasis [26]. The majority of authors suggested that deficiency in several nutrients such as proteins, minerals and vitamins affects bone metabolism, especially in vegetarians [19,27,28]. Our vegetarian children compared with omnivores had lower (by about 30%) protein intakes, expressed both as percentage of energy and as grams per kilogram of body weight. However, this intake met the recommended Polish data. As expected, in vegetarian diet the intake of animal protein was lower and plant protein higher compared with omnivorous diet. The ratio of animal protein to plant protein was above 3-fold lower in vegetarian diet than in omnivorous diet. Proteins have a number of potential positive and negative effects on bone health. Higher dietary protein intake may improve calcium absorption, osteoblast activity and promote mineralization of bone matrix. On the other

Table 5
Spearman correlations between CTX, OC and its fractions (c-OC and uc-OC) and dietary nutrient intakes unadjusted and adjusted for total energy intake in the group of vegetarian children.

	CTX		OC		c-OC		uc-OC	
	r	p	r	p	r	p	r	p
Dietary intake								
Total energy	–0.079	0.644	0.299	0.072	–0.016	0.924	0.282	0.090
% energy from protein	0.032	0.851	–0.080	0.636	0.256	0.126	–0.037	0.827
% energy from fat	–0.164	0.333	0.003	0.987	–0.066	0.699	0.095	0.578
% energy from carbohydrates	0.104	0.541	0.010	0.954	–0.053	0.755	–0.094	0.580
Total protein	–0.053	0.754	0.181	0.285	0.107	0.527	0.191	0.258
Animal protein	–0.153	0.366	–0.015	0.930	–0.037	0.828	0.103	0.545
Plant protein	–0.191	0.258	0.268	0.109	0.136	0.421	0.225	0.182
Calcium	0.046	0.786	–0.056	0.743	0.065	0.702	0.009	0.956
Phosphorus	–0.014	0.937	0.224	0.183	0.167	0.324	0.228	0.176
Magnesium	–0.075	0.660	0.266	0.112	0.214	0.205	0.182	0.282
Vitamin D	0.107	0.529	–0.034	0.839	–0.163	0.336	–0.016	0.927
Fiber	–0.119	0.483	0.367	0.025	0.314	0.059	0.339	0.040
Energy adjusted intake								
Total protein	0.027	0.874	–0.132	0.437	0.246	0.143	–0.065	0.702
Animal protein	0.042	0.803	–0.138	0.416	0.022	0.898	–0.023	0.890
Plant protein	–0.106	0.534	0.024	0.889	0.366	0.026	–0.070	0.679
Calcium	0.237	0.157	–0.236	0.160	0.042	0.806	–0.140	0.407
Phosphorus	0.078	0.648	–0.058	0.732	0.348	0.035	–0.013	0.937
Magnesium	0.037	0.830	0.103	0.545	0.486	0.002	–0.042	0.803
Vitamin D	0.119	0.484	–0.044	0.797	–0.122	0.473	–0.044	0.798
Fiber	0.031	0.855	0.267	0.110	0.488	0.002	0.227	0.177

CTX – C-terminal telopeptide of collagen type I, OC – osteocalcin, c-OC – carboxylated osteocalcin, uc-OC – undercarboxylated osteocalcin.

hand, high protein intake increases dietary acid load, especially with a higher intake of sulfur-containing amino acids which is associated with high bone resorption [18]. It is worth to note that adequate amount and type of protein from a variety of sources is important for maintenance of bone health, especially in growing children.

Sufficient intake and good absorption of bone forming minerals (Ca, P, Mg) and vitamins (vit D, vit K) may contribute to proper skeletal growth and development [21,29,30]. Calcium is a very important nutrient in bone health because nearly 99% of the calcium in the human body is contained in bones as hydroxyapatite. Vitamin D is the nutrient, which is involved in bone metabolism, modulating expression of osteocalcin gene through its nuclear receptor (VDR). Besides this, vitamin D regulates OC gene at post-translational level by stabilizing the osteocalcin mRNA [31]. On the contrary, vitamin K plays a significant role in the process of osteocalcin carboxylation [8].

Our studied children (both vegetarian and omnivores) had adequate intakes of phosphorus, magnesium and fiber. However, only 53% of vegetarians and 62% omnivores met recommended intake regarding calcium and 40% vegetarians and 42% omnivores regarding vitamin D. Moreover, bioavailability of these nutrients from plant sources is lower than from animal products. The studied children were following a lacto-ovo-vegetarian diet, which includes dairy products and eggs and that is why their dietary intake of calcium and vitamin D was comparable with omnivores. Adequacy of vitamin D can also be assessed by measuring serum 25-hydroxyvitamin D (as a marker of vitamin D status). Despite similar mean levels of this vitamin in both groups of children, 26% of vegetarian children and 28% of omnivores had an optimal level of serum vitamin D. We suggested that the intake of calcium and vitamin D in prepubertal children should be under special attention, not only in vegetarians, but also in omnivores.

So far, the reports on bone mineral density in adult vegetarians are contradictory. Some studies found no significant differences in BMD between vegetarians and non-vegetarians [32–34] while other found a decrease in the lumbar spine BMD [35] or in the femoral BMD [36]. Meta-analyses conducted by Ho-Phan et al. [37] showed that vegetarian adults had about 4% lower lumbar spine BMD than omnivores. Generally, researchers confirmed that lacto-ovo-vegetarians and omnivores have similar BMD, however, subjects on a restricted vegan diet have lower BMD and an elevated risk of osteoporosis [33,38]. The data concerning bone mineral density in children are limited [28,39]. In our prepubertal lacto-ovo-vegetarian children, we observed significantly lower z-scores of Δ TBLH-BMD and Δ BMDL2-L4 in vegetarians compared with omnivores which positively correlated with anthropometric variables but not with dietary nutrient intakes, serum adipokines, and bone turnover markers. The studied children were in prepubertal period and even slight changes in bone metabolism can have impact on their bone health in later life. The goal is to create the best possible condition for them to achieve optimal peak bone mass.

Recent studies demonstrate that adipokines (leptin, adiponectin) are contributors to the fat–bone relationship due to their association with body weight [40,41]. Leptin might influence bone in two opposing ways. It can promote bone formation locally through direct binding of leptin receptors in primary osteoblasts or may cause bone resorption and osteoclast activation via centrally mediated sympathetic pathway [42,43]. Also, adiponectin appears to be one of a growing number of nutrition-related peptides that impact bone mass. The receptors for adiponectin have been identified on both osteoblasts and osteoclasts which suggests a potential direct influence of this hormone on bone [44]. The final sum of direct and indirect actions of adiponectin on bone mass seems to be

negative and clinical studies demonstrate that its serum level is inversely related to BMD.

It is known that serum leptin level is influenced by body fat, being lower in lean and higher in overweight and obese individuals. We observed lower median value of leptin in vegetarian compared with omnivorous children but after adjusting for the percentage of fat mass, this significant difference disappeared between vegetarians and omnivores. We determined serum leptin levels for omnivorous children at the level of median value about 3 ng/mL. This level was similar to the one achieved in our previous studies [45,46], but higher than achieved in the study conducted by Erhardt et al. [47]. The observed difference in leptin levels in healthy children might result from the fact that our studied children had a higher percentage of fat mass (22% in girls and 19.5% in boys) than in the IDEFICS study (17% in girls and 15% in boys). The lack of correlations between adipokines and bone mineral density in our studied children did not prove their role in bone metabolism in these subjects.

In the existing literature, there were few reports describing levels of bone turnover markers in adult vegetarians. The authors found similar values of OC, BALP, and CTX and concluded that there was no evidence of increased bone turnover in subjects following vegetarian diets [35,36]. Little is known about bone metabolism markers in children and adolescents following vegetarian diets [48]. In present research, we observed similar levels of bone formation markers and a tendency to higher levels of bone resorption markers. Decreased ratio of OC to CTX and increased serum levels of parathormone in vegetarians compared with omnivores, might suggest elevated bone resorption. On the other hand, we found higher ratios of c-OC/uc-OC as well as c-OC/total OC in children on vegetarian diet than in meat eaters, which may be related to higher vitamin K intake in vegetarian diet rich in fruits and vegetables.

Carboxylated form of OC is essential for bone metabolism, although precise mechanisms have not been fully understood. The gamma-carboxylation of OC induces the conformational changes in molecules and confers greater affinity for calcium. The failure of carboxylation results in increased uc-OC, with less affinity to calcium ions and consequently reduced bone quality [10]. Low c-OC (possibly as a result of inadequate vitamin K status) may be an effect of a decrease in osteocalcin γ -carboxylation, and in consequence elevated proportion of uc-OC. In adults, Luukinen et al. [49] and Gundberg et al. [9] observed that high percentage of uc-OC was associated with greater risk of low BMD and the occurrence of fractures. There is little information about serum c-OC and uc-OC levels in pediatric population. Tubic et al. [50] analyzed total OC and its forms in normal-weight and overweight children and found significantly lower c-OC level in overweight subjects than in the controls. Prats-Puig et al. [51] observed higher uc-OC to c-OC ratio in leaner children than in the heavier ones.

We assessed for the first time serum levels of carboxylated and undercarboxylated osteocalcin in children following vegetarian diet and observed significantly higher ratio of c-OC to uc-OC than in omnivorous children. Although OC and its forms did not correlate with anthropometric parameters and other biochemical markers, we found significant positive correlations between c-OC and dietary intake (after adjustment for total energy) of plant protein, fiber, phosphorus and magnesium. For vegetarians, although many nutrients require attention to achieve adequacy (calcium, vitamin D), good-quality vegetarian diets include high intake of several important nutrients that may protect bone (alkalizing food, magnesium, vitamin K).

This study has some limitations. Firstly, the cross sectional study design was an important limitation. Secondly, our study sample size was relatively small which lacks sufficient power to detect moderate associations with statistical significance. However,

groups of children on vegetarian and omnivorous diets were comparable in terms of ethnicity (all participants were Caucasian), age, sex, weight, height, and BMI. Additionally, all vegetarians were following lacto-ovo-vegetarian diet from birth. Thirdly, we did not assess dietary vitamin K intake and did not measure serum vitamin K level directly, but we determined carboxylated and under-carboxylated forms of osteocalcin as well as the ratio of c-OC/uc-OC.

In conclusion, prepubertal children on a vegetarian diet had significantly lower total and lumbar spine BMD z-scores, but absolute values of bone mineral density did not differ. BMD z-scores did not correlate with bone metabolism markers and nutritional variables, but were positively associated with anthropometric parameters. Lower leptin levels in vegetarian children reflect lower body fat. Longitudinal studies are necessary to evaluate the impact of the observed association on bone health at adulthood.

Statement of authorship

The authors have equally contributed to the realization of this manuscript.

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Conflict of interest

The authors declare that they do not have any conflicts of interest.

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