Serum concentration of biochemical bone turnover markers in vegetarian children

Ambroszkiewicz J[1]*, Klemarczyk W[2], Gajewska J[1], Chełchowska M[1], Laskowska-Klita T[1]

[1] Department of Biochemistry, Institute of Mother and Child, Warsaw, Poland
[2] Department of Nutrition, Institute of Mother and Child, Warsaw, Poland

Abstract

Purpose: In general, most children on well-planed vegetarian diets can achieve normal growth and development. However, elimination of animal products from the diet decreases the intake of some essential nutrients, such as calcium and vitamin D, and may influence bone metabolism. This is especially important in childhood and adolescence, when growth and bone turnover are most intensive. The aim of this study was to investigate the serum concentrations of biochemical bone turnover markers in prepubertal vegetarian children.

Material and methods: We examined 50 children on vegetarian and 50 on omnivorous diets aged 2-10 years. Dietary constituents were analyzed using a local nutritional program. Serum bone formation (OC, BALP) and resorption (CTX) markers were determined by specific enzyme immunoassays (ELISA) and 25-hydroxyvitamin D by the chemiluminescence method (CLIA).

Results: The average daily energetic value and the percentage of energy from protein, fat and carbohydrates in the diets were similar in both groups of children and were within the recommended range. The vegetarian children showed about a two-fold lower daily intake of calcium and vitamin D than their omnivorous counterparts. The level of 25-hydroxyvitamin D in the serum of vegetarian children was also nearly 2-fold lower compared with omnivores. In vegetarians, as compared to non-vegetarians, mean serum concentrations of OC, BALP and CTX were lower by about 20%, 10% and 15%, respectively.

Conclusion: Our preliminary results suggest that an inadequate dietary intake of calcium and vitamin D may impair bone turnover rate in vegetarian children. The parameters of bone metabolism should be monitored in these children in order to prevent bone abnormalities.

Key words: vegetarian diets, children, bone turnover markers, vitamin D.

Introduction

Vegetarian diets can be healthy when they are well balanced and if a variety of foods is consumed [1-3]. However, vegetarian diets with exclusion of nutrients from animal foods may influence bone metabolism, especially in childhood and adolescence when growth and bone turnover are most intensive [4-7]. Some authors investigating adult vegetarians have described that decreased calcium and vitamin D intake resulted in lower plasma vitamin D concentration and bone mineral density (BMD) [8-11]. Apart from measuring bone mineral content and density, biochemical bone turnover markers showing global skeletal activity have lately been developed and validated for the assessment of the dynamics of bone formation and resorption processes. Among them, products of the osteoblast activity (osteocalcin – OC, bone alkaline phosphatase – BALP), which are markers of bone formation, and products of osteoclast activity as markers of bone resorption (collagen type I terminal telopeptide – CTX) are considered to be clinically useful [12,13].

Osteocalcin, the major non-collagenous protein, synthetized by osteoblasts plays an important role in the regulation of bone growth and in the correct deposition of the minerals in the matrix. Its expression follows the proliferative phase of osteoblastic differentiation, so it can be considered a marker of mature osteoblasts. Bone alkaline phosphatase located in the plasma membrane of osteoblasts is one of the isoenzymes of alkaline phosphatase which plays an active role in bone formation and

* CORRESPONDING AUTHOR:
Department of Biochemistry
Institute of Mother and Child
ul. Kasprzaka 17a, 01-211 Warszawa, Poland
Tel: +48 22 3277260, Fax: +48 22 3277280
e-mail: jagodam7@yahoo.com (Jadwiga Ambroszkiewicz)

Received 11.05.2007 Accepted 02.07.2007

skeletal mineralization. BALP appears in the matrix maturation phase and is a marker of the middle stage of bone formation. During bone resorption, a molecule of collagen is degraded and small fragments are liberated into the blood-stream. The 8-amino acid sequence (Glu-Lys-Ala-His-Asp-Gly-Gly-Arg) found in the C-terminal telopeptide of the α1 chain of type I collagen, which can undergo beta-isomerization, has proven to be a sensitive marker of the bone resorption process.

Serum levels of OC, BALP and CTX are not stable throughout life and are greater in infants and children than in adults. Peak values occur at puberty. Children have significantly elevated bone marker levels due to high skeletal growth velocity and rapid bone turnover during childhood growth [14,15]. Many physiological and pathological processes may influence bone metabolism resulting in changes in serum concentration of bone turnover markers. Measurements of these parameters offer many advantages for investigating skeletal diseases in children and adolescents as well as monitoring the response to treatment. Little is known regarding bone metabolism status in children on vegetarian diets.

The aim of this study was to assess serum concentrations of biochemical bone formation and resorption markers in prepubertal vegetarian children.

Material and methods

We examined 100 healthy prepubertal children with two different nutritional habits (vegetarian and omnivorous diet) who had been referred during wintertime (from November to February) to the Institute of Mother and Child (Warsaw). None of the subjects was receiving any medications or had a history of metabolic bone disease or any serious health problem. The whole group of investigated children was ethnically homogenous. Among them 50 children (23 girls, 27 boys) aged 2-10 years presented at the Department of Nutrition for dietary consultation were vegetarians. In this group there were 28 lacto-ovo-vegetarians (did not consume meat, poultry, fish, but ate eggs and dairy products), 4 lacto-vegetarians (excluded eggs), 5 ovo-vegetarians (excluded milk products, but ate eggs) and 13 vegans (excluded all foods of animal origin).

Healthy children (n=50; 25 girls, 25 boys), range age 2-10 years on an omnivorous diet sent to our laboratory for routine analytical control were the reference group. The study was approved by the Ethics Committee of our institution and informed consent was obtained from parents of the examined children. Dietary constituents (especially calcium, phosphate, vitamin D) and nutrient supplementation data were assessed by questionnaire and calculated using the local nutritional computer program (Dieta2®, National Food and Nutrition Institute, Warsaw).

Venous blood samples were obtained from fasting patients in the morning (8-10). Serum was prepared by centrifugation (1000 g for 15 min at 4°C) and concentrations of calcium and phosphate were determined by colorimetric methods with commercially available kits from Hoffman-La Roche (Switzerland) on Cobas Integra analyzer. Remaining serum samples were frozen at -20°C for the analysis of bone turnover markers and vitamin D within 2 months. Serum OC was analyzed immunoenzymatically using the N-Mid Osteocalcin ELISA kits (Nordic Bioscence Diagnostics, Denmark) which is based on the application of two highly specific monoclonal antibodies against human OC by recognising the midregion (20-29 aa) and the N-terminal region (10-16 aa) of osteocalcin. The sensitivity of this assay is 0.5 µg/L, the intraassay imprecision (CV) – 3.4% and the interassay CV – 6.4%. BALP activity was evaluated by a specific enzyme immunoassay utilising a monoclonal anti-BALP antibody coated on the strip to capture BALP in the sample (Alkphase-B kit, Metra Biosystems, San Diego, USA). The sensitivity of this assay is 0.7 U/L and the intra- and interassay CVs are below 2.7%. Serum CTX was determined using the Serum CrossLaps ELISA kits (Nordic Bioscence Diagnostics, Denmark). This assay is based on monoclonal antibodies that recognise the beta-aspartate isomerized form of the sequence (Glu-Lys-Ala-His-Asp-Gly-Gly-Arg) derived from the C-telopeptide region of the type I collagen alfa1-chain. According to the manufacturer, the intra- and interassay imprecision (CVs) are less than 8.1% and the lower detection limit is 0.9 ng/mL. Serum 25-hydroxyvitamin D was determined by chemiluminescence immunoassay (CLIA) using (DiaSorin kits, Stillwater, USA). During the incubation, 25-OH vitamin D is dissociated from its binding protein, and competes with labelled vitamin D for binding sites on the antibody. The detection limit of the assay is 7.0 ng/mL, the intra- and interassays CVs are 6.4% and 3.4%.

All data were presented as mean value ± standard deviation (SD). The Statistica (version 6.0) computer software was used for statistical analysis. The differences were regarded as statistically significant at $p<0.05$.

Results

Vegetarian children were in the same age and had similar average Body Mass Index (BMI) 15.6±1.4 kg/m^2 as their omnivorous counterparts 16.0±1.3 kg/m^2. Mean daily energy intake and the percentage of energy from protein, fat and from carbohydrates were within the reference range and similar in both groups (*Tab. 1*). The daily intakes of calcium and phosphate of omnivorous children were in the recommended range [16]. In vegetarian children the intake of phosphate was adequate and calcium was below the recommended range. Dietary intake of vitamin D in both groups of the tested children was very low in according to the recommendations. In vegetarian children vitamin D intake was about two-fold lower than in omnivores ($p<0.001$).

Concentrations of calcium and phosphate in serum were in the physiological range in all tested children, but the 25-OH vitamin D level in vegetarians was two-fold lower as compared to that in non-vegetarians ($p<0.0001$) (*Tab. 2*). The mean serum levels of all measured biochemical bone turnover markers were significantly lower (OC by about 20%, BALP – 10% and CTX – 15%) in vegetarian children in comparison with omnivores.

Table 1. **Average daily energy and nutrients intake of examined children compared to recommended daily intake**

	Vegetarian children	Omnivorous children	Recommended daily intake*
Energy values (kcal)	1 468±409	1 591±305	1 400-1 700
Energy from protein (%)	13.1±2.5	14.2±3.1	12.0-14.0
Energy from fat (%)	30.1±5.9	30.5±6.5	32.0
Energy from carbohydrates (%)	56.2±5.9	55.3±7.1	56.0-58.0
Dietary Ca (mg)	559±345	821±335*	800-1 000
Dietary P (mg)	910±337	949±294	800-1 000
Dietary vitamin D (µg)	1.37±1.10	2.43±1.38*	10.0

Data are shown as mean values ± SD, * p<0.001

* Szotowa W, Socha J, Charzewska J, Dłużniewska K, Jabłoński E, Kunachowicz H, Rudzka-Kańtoch Z, Ryżko J, Stolarczyk A, Weker H. Dzienne zalecenia żywieniowe dla dzieci i młodzieży. Ped Pol, 1995; 1: 1-12.

Table 2. **Serum calcium, phosphate, vitamin D and bone turnover marker concentrations in vegetarian and omnivorous children**

	Vegetarian children	Omnivorous children
Ca (mmol/L)	2.35±0.12	2.39±0.15
P (mmol/L)	1.60±0.19	1.73±0.17
25 OH vitamin D (ng/mL)	13.9±7.2	29.8±4.7***
OC (µg /L)	71.0±20.1	88.9±17.5**
BALP (U/L)	94.5±21.5	104.5±28.4*
CTX (ng/L)	1 697±653	1 993±300*

Data are shown as mean values ±SD; * p<0.01, ** p<0.001, *** p<0.0001

Discussion

Adequate and appropriate nutrition is important for all individuals, but not all follow a diet that is optimal for bone health. Calcium and vitamin D are the specific nutrients most important for attaining peak bone mass and for preventing osteoporosis [17]. Besides the amount of calcium in the diet, its absorption is also a critical factor in determining the availability of calcium for bone development and maintenance. Vegetarians who consume milk products have intakes of calcium as high as those of omnivores, but strict vegetarians are at risk of calcium and vitamin D deficiency [8]. In vegan diets care must be taken to choose plant foods that are high in calcium, another option is calcium-fortified food or supplementation.

In our vegetarian children the daily intakes of calcium and vitamin D were below the recommended values. Serum concentrations of calcium in both groups of children were within the physiological range but vitamin D was two-fold lower in vegetarians. Childhood and adolescence are very important periods for bone metabolism because most of the peak bone mass is accumulated during these years. Deficiencies in some of the nutrient components (calcium, vitamin D) together with reduced serum concentration of vitamin D may retard relevant bone growth and development. Research data available show that bone mineral density of the lacto-ovo-vegetarian children was comparable to the general omnivorous population [18]. However, adolescents who consumed a vegan diet in early life, demonstrated a lower relative bone mass than their omnivorous counterparts [19]. Data from the existing literature regarding adult individuals on vegetarian diets (especially vegans) suggest that low BMD at clinically important skeletal regions was present [20]. According to other authors normal bone mass in vegetarians, especially in lacto-ovo-vegetarians was also observed [21].

To our knowledge there are only two reports presenting values of biochemical bone turnover markers in vegetarian subjects. Fontana et al. [20] in their study observed similar levels of CTX and BALP between adult omnivores and 18 vegetarians (54.2±11.5 years) consuming their respective diet for 3.6 years on average. In adolescent vegetarians (aged 9-15 years) Parsons et al. [22] found also similar values of bone metabolism parameters as in omnivores. There are no studies regarding bone metabolism markers in prepubertal children on vegetarian diets. In our study we detected significantly reduced concentrations (by about 10-20%) of serum bone metabolism markers in vegetarians compared with omnivores. The examined vegetarian children were on different kinds of diet, but mean values of bone turnover markers for the vegans and lacto-ovo-vegetarians were not significantly different.

In conclusion, our results suggest that an inadequate dietary intake of calcium and vitamin D may impair bone turnover rate in vegetarian children. Further studies of bone metabolism in children following vegetarian diets with vitamin and mineral supplementation are needed; this data may assist in deciding whether supplementation is useful and help to prevent bone abnormalities in their later life.

References

1. Rajaram S, Sabate J. Health benefits of vegetarian diet. Nutrition, 2000; 16: 531-3.
2. Mangels AR, Messina V, Melina V. Position of the American Dietetic Association and Dietitions of Canada: vegetarian diets. J Am Diet Assoc, 2003; 103: 748-65.
3. Leitzmann C. Vegetarian diets: what are the advantages? Forum Nutr, 2005; 57: 147-56.
4. Key TJ, Appleby PN, Rosell MS. Health effects of vegetarian and vegan diets. Proc Nutr Soc, 2006; 65: 35-41.
5. Sauders TA. Vegetarian diets and children. Pediatr Clin North Am, 1995; 42(4): 955-65.

6. Thane CW, Bates CJ. Dietary intakes and nutrient status of vegetarian preschool children from a British national survey. J Hum Nutr Diet, 2000; 13: 149-62.

7. Dunham L, Kollar LM. Vegetarian eating for children and adolescents. J Pediatr Health Care, 2006; 20(1): 27-34.

8. Lamberg-Allardt C, Karkkainen M, Seppanen R, Bistrom H. Low serum 25-hydroxyvitamin D concentrations and secondary hyperparathyroidism in middle-aged white strict vegetarians. Am J Clin Nutr, 1993; 58(5): 684-9.

9. Lapatsanis D, Moulas A, Cholevas V, Soukakos P, Papadopoulou ZL, Challa A. Vitamin D: a necessity for children and adolescents in Greece. Calcif Tissue Int, 2005; 77(6): 348-55.

10. Outila TA, Karkkainen MU, Seppanen RH, Lamberg-Allardt CJ. Dietary intake of vitamin D in premenopausal, healthy vegans was insufficient to maintain concentrations of serum 25-hydroxyvitamin D and intact parathyroid hormone within normal ranges during the winter in Finland. J Am Diet Assoc, 2000; 100(4): 434-41.

11. Chechowska M, Ambroszkiewicz J, Gajewska J, Klemarczyk W, Laskowska-Klita T. Influence of vegetarian diet on fat-soluble vitamins (A, D, E) in prepubertal children. Polish J Environ Stud, 2006; 15(5b): 586-9.

12. Calvo MS, Eyre DR, Gundberg CM. Molecular basis and clinical application of biological markers of bone turnover. Endocrine Rev, 1996; 17: 333-68.

13. Schulc P, Seeman E, Delmas PD. Biochemical measurements of bone turnover in children and adolescents. Osteoporosis, 2000; 11: 281-94.

14. Yang L, Grey V. Pediatric reference intervals for bone markers. Clin Biochem, 2006; 39(6): 561-8.

15. Gajewska J, Ambroszkiewicz J, Laskowska-Klita T. Wybrane markery obrotu kostnego w surowicy krwi dzieci w zależności od wieku i płci. Wiad Lek, 2005; 58(9-10): 476-80.

16. Szotowa W, Socha J, Charzewska J, Dłużniewska K, Jabłoński E, Kunachowicz H, Rudzka-Kańtoch Z, Ryżko J, Stolarczyk A, Weker H. Dzienne zalecenia żywieniowe dla dzieci i młodzieży. Ped Pol, 1995; 1: 1-12.

17. Cashman KD. Calcium intake, calcium bioavailability and bone health. Br J Nutr, 2002; 87, (Suppl. 2):169-77.

18. Leung SS, Lee RH, Sung RY, Luo HY, Kam CW, Yuen MP, Hjelm M, Lee SH. Growth and nutrition of Chinese vegetarian children in Hong Kong. J Paediatr Child Health, 2001; 37(3): 247-53.

19. Dhonukshe-Rutten RA, van Dusseldorp M, Schneede J, de Groot LC, van Staveren WA. Low bone mineral density and bone mineral content are associated with low cobalamin status in adolescents. Eur J Nutr, 2005; 44(6): 341-7.

20. Fontana L, Shew JL, Holloszy JO, Villareal DT. Low bone mass in subjects on a long-term raw vegetarian diet. Arch Intern Med, 2005; 165(6): 684-9.

21. Siani V, Mohamed EI, Maiolo C, Di Daniele N, Ratiu A, Leonardi A, De Lorenzo A. Body composition analysis for healthy Italian vegetarians. Acta Diabetol, 2003; 40 (Suppl 1): 297-8.

22. Parsons TJ, van Dusseldorp M, Seibel MJ, van Staveren WA. Are levels of bone turnover related to lower bone mass of adolescents previously fed a macrobiotic diet? Exp Clin Endocrinol Diabetes, 2001; 109(5): 288-93.