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Intake of Calcium and Phosphorus and Levels of Bone Mineralization (BMC) and Mineral Bone Density (BMD) of Female Swimmers in the Pubescence Period

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The objective of this study was to evaluate bone mineralization (BMC) and bone mineral density (BMD) of the osseous tissue in girls training swimming an being in the period of reaching the peak bone mass, as compared to girls being at a similar age and non-practicing sport, taking into account dietary allowances for calcium and phosphorus and dietary ratios of these elements.

Both the swimmers and their non-training colleagues were found to meet nutritional demands to the same extent and their diets did not differ in the intakes of energy nor nutrients (protein, calcium, phosphorus), which is incorrect in the case of the non-training girls. An alarmingly low intake of calcium at a, simultaneously, excessive intake of protein and phosphorus, as well as incorrect ratios between calcium and phosphorus and between calcium and protein observed especially in the case of the swimmers, might have an adverse effect on the mineralization of osseous tissue in the period of reaching peak bone mass.

INTRODUCTION

Most of investigations conducted so far confirm the fact that going in for sport in the childhood and adolescence exerts a highly favourable effect on the osseous tissue [Gustavsson *et al*., 2003; Johannsen *et al*., 2003; Nurmi-Lawton *et al*., 2004]. Zanker *et al*. [2004] report that high mineralization of osseous tissue reached by female gymnasts during sports career was sustaining also after its termination. Apart from physical activity, the development and stabilization of osseous tissue is significantly affect by many environmental factors, including eating habits with appropriate intake of calcium in particular. Unfortunately, many research have demonstrated a low content of this element in diet of schoolchildren, which is the likely cause of the unsatisfactory mineralization of man's skeleton both in the period of development and in later stages of life [Błaszczyk & Chlebna-Sokół, 2003; Górecka *et al*., 2000].

The objective of this study was to evaluate bone mineralization (BMC) and bone mineral density (BMD) of the osseous tissue in girls training swimming and being in the period of reaching the peak bone mass, as compared to girls being at a similar age and non-practicing sport, taking into account dietary allowances for calcium and phosphorus and dietary ratios of these elements in diet.

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MATERIALS AND METHODS

The study covered 32 girls at the age of 11–15 years, attending sports schools (Ożarów Mazowiecki and Międzyrzec Podlaski) and training swimming for 2.3±1.2 years. The girls represented a high sports level which was indicated by their participation in competitions at a national level (Championships of Poland). A control group was constituted by 24 non-training girls at a similar age, attending the same classes. The study was conducted in June and September of 2008. It was approved by the Bioethical Commission of the Academy of Physical Education in Warsaw.

Somatic traits of the girls were evaluated based on measurements of their body height (exact to 0.1 cm), body mass (exact to 0.1 kg), and thickness of skinfolds on triceps (TST), abdomen (AST) and calf (CST). Skinfold thickness was measured exact to 0.1 mm on the right side of the body using a Harpenden skinfold calliper. All measurements were taken by a trained person. In addition, calculations were made for body mass index – BMI (body mass/body height^2; kg/m^2) and for the percentage content of fatty tissue in the body with the method by Slaughter *et al*. [1988].

Bone mineralization (BMC, g) and density (BMD, g/cm^2) were determined in the lumbar section of the spine (L_2–L_4) with dual-emission X-ray absorptiometry using a Lunar DPX–L apparatus (USA). In addition, a reference paediatric data base for the densytometric apparatus provided information on relative bone mineral density BMD (%) of each child, being a percentage of bone mineral density in respect of children from the reference group and Z-score being a standard deviation from the reference value expressed by the number of standard deviations SD [Górecka *et al*., 2000].

Eating habits of the girls were evaluated based on 3 questionnaire recalls of the 24 hours preceding the study. The size of food rations consumed was evaluated using "Album of Photographs of Products and Dishes" [Szponar *et al*., 2008]. Intakes of energy, protein, calcium and phosphorus from diet were calculated using a computer software based on national tables of food composition and nutritive value [Kunachowicz *et al*., 2005]. Results were presented in the form of median (Me) and quartile deviation (QD) being half the difference between the third and the first quartile. Results obtained for the intakes protein and phosphorus were compared with estimated average requirements (EAR) for a group, whereas these for the intake of calcium – with adequate intakes (AI), considering: sex, age, body mass and physical activity in the case of energy; sex, age and body mass in the case of protein; and sex and age in the case of Ca and P [Jarosz & Bułhak-Jachymczyk, 2008]. In the case of energy, the evaluation of its AI was conducted based on BMI standards for children and adolescents [Palczewska & Niedźwiecka, 2001] adopting the following cut-off points:
– normal energy intake: BMI between 25 and 75 ptc,
– insufficient energy intake: BMI up to 25 ptc,
– excessive energy intake: BMI over 75 ptc.

In order to estimate the insufficient intakes of protein and phosphorus, calculations were made for the percentage (%) of subjects whose diets did not cover the estimated average requirements (EAR) of the group. In subjects with a low intake of these elements (below EAR), the likelihood of the occurrence of a high risk of insufficient intake exceeds 50%. In turn, intakes above EAR indicate that the likelihood of the correct intake of a nutrient is higher than 50% [Murphy *et al.*, 2006]. In order to detect a low risk of insufficient calcium intake, calculations were made for the percentage (%) of subjects in whom the intake of this element exceeded the adequate intake (AI). Further calculations were carried out for nutrient density of diet for calcium and phosphorus as well as for Ca:protein and Ca:P ratios that were next compared with recommended values computed based on dietary guidelines [Jarosz & Bułhak-Jachymczyk, 2008].

Differences between the median of energy intake in particular BMI percentile ranges in the group of girls training swimming and in the control group were evaluated with the Kruskal-Wallis test, whereas differences between the anthropometric and densytometric parameters were evaluated with the Student's t-test for independent variables, assuming a significance level of $p<0.05$ as statistically significant.

RESULTS

Data presented in Table 1 demonstrate that the swimmers were significantly ($p<0.05$) younger (11.5±0.9 years) than the girls from the control group (12.1±0.8 years). In addition, the swimmer displayed a tendency for a lower body mass (40.6±8.8 kg) and body height (151.7±9.5 cm) compared to the non-training girls (45.0±10.3 kg and 154.3±9.5 cm, respectively) as well as for a lower content of adipose tissue in the body (25.5±8.2% *vs*. 30.0±9.0%) and lower BMI values (17.4±2.3 kg/m^2 *vs*. 18.7±3.5 kg/m^2).

Data collated in Table 2 show that energy intake was the highest in the group with BMI values below the 25th percentile (the swimmers: 2282±253 kcal; the non-training girls: 2169±114 kcal). In the successive percentile ranges of BMI, the intake of energy was observed to decrease significantly ($p<0.05$) reaching the lowest values in the group of girls with BMI above the 75th percentile, *i.e.* 1953±121 kcal and 1801±172 kcal, respectively. Protein and phosphorus intakes from diet were alike in both groups compared, *i.e*. 64.4±12.2 g *vs*. 64.8±8.4 g and 1121±245 mg *vs*. 1044±156 mg for the training and non-training girls, respectively.

TABLE 1. Characteristics of the surveyed group of girls (mean±SD).

Group	Age (years)	Body mass (kg)	Body height (cm)	Fatty tissue content (%)	BMI (kg/m^2)	Physical activity (hours/week)
Girls training swimming (n=32)	11.5±0.9	40.6±8.8	151.7±9.5	25.5±8.2	17.4±2.3	12.1±3.3
Non-training girls (n=24)	12.1±0.8**	45.0±10.3	154.3±9.5	30.0±9.0	18.7±3.5	2.3±2.2

**$p<0.01$ – a significantly higher value from the respective value for girls training swimming (Student's t-test).

TABLE 2. Daily intake of energy, protein, calcium and phosphorus by the surveyed girls (Me±QD).

Group	Energy (kcal/day) BMI percentile ranges ≤ 25	Energy (kcal/day) BMI percentile ranges 25–75	Energy (kcal/day) BMI percentile ranges ≥ 75	Protein (g/day)	Phosphorus (mg/day)	Calcium (mg/day)
Girls training swimming (n=32)	2282±253A* (18.8%)**	1969±160B (65.6%)	1953±121BC (15.6%)	64.4±12.2 (0%)***	1121±245 (46.9%)	514±136 (0%)
Non-training girls (n=24)	2169±114A (25%)	2155±327AB (41.7%)	1801±172C (33.3%)	64.8±8.4 (0%)	1044±156 (58.3%)	432±172 (0%)

* median values with different letter differ significantly at p≤0.05; **percentage of girls in BMI percentile ranges; ***values in brackets provided in columns for protein and phosphorus indicate percentage of girls whose food rations failed to meet estimated average requirement (EAR), in the case of calcium the values in brackets indicate percentage of girls in whom the intake of this elements exceeded the adequate intake (AI) value.

TABLE 3. Nutrient density (Me±QD) and quantitative ratio of calcium to phosphorus and protein tin diet of the surveyed girls.

Group	Calcium		Phosphorus		Ca:P	Ca: Protein
	(mg/1000 kcal)	(mg/MJ)	(mg/1000 kcal)	(mg/MJ)	(mg:mg)	(mg/g)
Girls training swimming (n=32)	242.7±63.8	58.0±15.3	518.3±61.8	118.5±14.2	1:2.2	7.8:1
	(45%)*		(124%)		1:0.8**	
Non-training girls (n=24)	224.7±64.5	53.7±15.4	502.9±63.0	120.2±15.1		6.7:1
	(42%)		(115%)		1:2.4	

*values in brackets provided in the column for calcium indicate the average percentage of covering nutrient density of diet calculated based on adequate intake (IA) guidelines, in the case of phosphorus the values in brackets refer to the average percentage of covering nutrient density of diet calculated based on estimated average requirements (EAR) of the group; ** recommended Ca: quantitative ration calculated based on dietary guidelines.

In both groups, there were no subjects whose diet failed to cover the EAR for protein, whereas in *ca*. 47% of the swimmers and in over 58% of the non-training girls the diet failed to cover group's EAR for protein. Both groups were characterised by particularly low intake of calcium. Its content in the diet of swimmers and non-training girls did not differ significantly and reached 514±136 and 432±172 mg/person/day, with the adequate intake recommended for that group being 1300 mg/person/day [Jarosz & Bułhak-Jachymczyk, 2008]. In both groups, there were no girls with Ca intake exceeding AI.

The nutrient density of diet calculated for calcium as compared to that calculated based on guidelines for adequate intake (AI) values was provided in Table 3. This value reached 48% and 34% in the case of the swimmers and non-training girls and did not differentiate significantly the groups compared. The nutrient density of diet calculated for phosphorus, referred to that computed based on guidelines for group's estimated average requirements (EAR), was considerably higher and reached *ca.* 124% in the swimmers and 115% in the non-training girls, and did not differentiate significantly the groups compared either. The calcium to phosphorus (C:P) ratio diverged from the recommended value of 1:0.8 computed based on dietary guidelines and accounted for 1:2.2 and 1:2.4 in the case of the training and non-training girls. In turn, the calcium to protein ratio reached 7.8:1 and 6.7:1, respectively.

Likewise in the case of nutritional parameters, also the average values of bone parameters (Table 4) were observed not to differentiate significantly the groups of girls. The mean values of BMC and BMD were insignificantly higher in the control group and reached: 26.7±7.7 g *vs*. 30.0±10.5 g and 0.87±0.13 g/cm² *vs*. 0.92±0.18 g/cm², respectively. When comparing BMD values obtained in the study to reference values, both groups were shown to be characterised by standard deviation not exceeding 2.5% on average and by a tendency for higher absolute BMD value in favour of the control group (97.1±10.3% *vs*. 98.1±15.7%). The mean value of Z–score for BMD did not differ significantly between the swimmers and the non-training girls and accounted for, respectively: –0.25±0.95SD and –0.15±1.46SD (normal value). However, when results were evaluated individually, incorrect values of Z–score in the range of 1SD to 2SD (Z–score <–1SD to –2SD), were noted in 13% of the swimmers and 8% of girls from the control groups, whereas in the range of (Z–score <–2SD) – in 6% and 17% of the training and non-training girls, respectively.

TABLE 4. Results of densytometric measurements of the surveyed girls (mean±SD).

Group	BMC (g)	BMD (g/cm²)	$BMD_{\%}$ (%)	$BMD_{z\text{-score}}$ (SD)
Girls training swimming (n=32)	26.7±7.7	0.87±0.13	97.1±10.3	– 0.25±0.95
Non-training girls (n=24)	30.0±10.5	0.92±0.18	98.1±15.7	– 0.15±1.46

$BMC_{(g)}$ – content of mineral compound in bone, $BMD_{(g/cm^2)}$ – absolute value of bone mineral density, $BMD_{(\%)}$ – bone density compared to the respective age group expressed in per cents, $BMD_{z\text{-score}}$ – standard deviation from reference mean value for the same sex and age expressed by the number of standard deviations SD.

In the case of the swimmers, the mean weekly number of training hours accounted for 12.1±3.3 h, whilst in the case of the non-training girls the mean weekly number of hours spent on physical activity reached 2.3±2.2 h. In addition, the study demonstrated that *ca*. 29% of the girls from the control group did not participate in any form of physical activity during the week (Table 1).

DISCUSSION

Apart from genetic factors, the proper increase of bone mass in the period of intensive development is affected by environmental factors linked with lifestyle, the most significant of which include physical activity [Peer, 2004; Schwarz, 2004; Vicente-Rodríguez, 2008] and eating habits, appropriate intakes of calcium and phosphorus with diet in particular [Matkovic *et al*., 2005; Lanou *et al*., 2005].

Longitudinal observations demonstrate that adults, especially women, who were active in the childhood and pubescence are characterised by a better status of the skeletal system. Corroborating evidence for the effect of physical exercises on the osseous tissue has been provided by investigations conducted in the pubescence period, *i.e.* during reaching the peak bone mass [Gustavsson *et al*., 2003; Johannsen *et al*., 2003; Nurmi-Lawton *et al*., 2004]. Increased physical activity has been implicated to enhance mineralization, and appropriately adjusted and systematic training has been shown to assure reaching high peak bone mass, which determines bone status in the later, adult life [Janz *et al*., 2004]. In a vast review work, Karlsson [2004] suggests for example that in the case of girls being in the pubescence period, sports training yields a higher peak bone mass than in the non-training girls. It has been confirmed by surveys conducted with sportsmen of different disciplines which indicate that the increased physical activity leads to a higher bone mineral density [Gustavsson *et al*., 2003; Johannsen *et al*., 2003; Nurmi-Lawton *et al*., 2004].

Analyses conducted in this study demonstrate that the mean values of BMC and BMD were insignificantly higher in the control group (Table 4). Also when compared to reference values, the absolute BMD value tended to be higher also in the control group (97.1±10.3% *vs.* 98.1±15.7%), despite a significantly ($p<0.001$) higher average number of hours devoted to trainings by the swimmers.

The lack of significant differences between the mean BMD and BMC values may be explained by, among other things, the type of physical activity. Taaffe *et al.* [1997] were explaining lower values of osseous parameters noted in female swimmers by the effort being typical of this sports discipline and not involving overcoming the gravity, *i.e.* effort made under conditions of a lesser effect of the gravity force. In turn Karlsson *et al*. [1993] claim the optimal form of physical activity having a beneficial effect on bone status to be resistant exercises. Their positive effects have been reported in women at the age of 16–21 years [Karlsson *et al*., 1993] and older [Mac Auley, 2001].

Investigations by Ward *et al*. [2007] demonstrate the positive effect of physical exercises only when coupled with a well-balanced diet assuring the adequate intake of energy, and especially of protein, calcium and phosphorus. The optimum intake of calcium and common ratios of calcium, phosphorus and protein are of great importance at each stage of man's life, however due to the realization of the genetic programme of the body in terms of bone mass building, they are of the outmost significance in the period of childhood and adolescence [Eastell & Lambert, 2002; Specker & Binkley, 2003].

Results of many authors point to the common ill-balancing of diets of schoolchildren. Surveys conducted by Ustynowicz-Fabiszewska *et al*. [2002] and Zagórecka *et al*. [2000] amongst children and adolescents from the north-eastern regions of Poland demonstrate a low intake of calcium reaching 43–60% and excessive intake of phosphorus exceeding 40% of RDA. Similar observations were made by Czeczelewski & Raczyńska [2005] who evaluated everyday food rations of 10–15 year old children from primary schools and junior secondary schools of the Bialski Province. The intake of calcium by girls and boys from age categories of 10–12 and 13–15 years accounted for 43% and 51% as well as 44% and 65% of RDA, whereas that of phosphorus exceeded the recommended level and reached 122% and 133% for girls and 141% and 165% for boys from the respective age categories.

The above-cited data on the alarmingly low calcium intake, at a simultaneously excessive intake of phosphorus, refer also to children training sports, which is confirmed by the reported study (Table 2). The latter demonstrates that diets of 47% of the girls training swimming and these of 58% of the non-training girls failed to meet the estimated average requirements (EAR) for phosphorus. The opposite case was with calcium – its adequate intake (AI) was not exceeded by any of the girls examined.

The high phosphorus intake is especially dangerous when coupled with a low calcium content in the diet. This disturbs the calcium to phosphorus ratio (Table 3), which in children's diet should range from 2:1 to 1:1 and may only be assured by a diet based on natural food products, including especially milk and dairy products [Rutkowska & Kunachowicz, 1994].

In an average European and American (USA) diet, the richest source of easily-available calcium are milk and dairy products. They are estimated to provide even up to 75% of this element [Cichy & Rosińska, 2005; Heaney, 2002a]. According to Bos *et al*. [2000] and Guéguen & Pointillart [2000], milk and dairy products contain a number of bioactive substances that exert a positive effect on the effectiveness of intestinal absorption of calcium. The significance of dairy products as the major source of calcium has been confirmed by longitudinal surveys of American girls [Fiorito *et al*., 2006]. They indicate that the observed higher intake of calcium at the age of 7 and 9 was positively correlated with higher BMC values noted at the age of 11. Also Moore *et al*. [2008] in the Framingham Children's Study demonstrated the beneficial effect of dairy products consumption in the childhood on higher BMC values in the adolescence. According to those authors, the eating habits including the habit of dairy products intake in the childhood, are likely to lead to their higher intake also in the adult life.

In contrast, the excessive intake of phosphorus is linked not only with the common occurrence of this element in food products, but also with addition of phosphates to food in order to extend its shelf life and improve its organoleptic characteristics [Rutkowska & Kunachowicz, 1994; Nowak, 2004]. According to Fenton *et al*. [2009], however, there is no evidence for the adverse effect of increased phosphates intake on the osseous tissue. The analysis of the above literature data indicates that the increased intake of phosphates was implicated to reduce Ca content in urine and to increase its retention. However, the meta-analysis failed to provide explicit evidence for phosphate intake contributing to enhanced excretion of calcium with urine and likely demineralization of the osseous tissue. According to those authors, dietetic recommendations emphasizing the adverse effect of phosphates contained in dairy products, meat and legumes should be re-evaluated.

It may be speculated that the contribution of milk and dairy products in diets of the surveyed girls was insufficient, which is corroborated by the low nutrient density of the diet computed for calcium, *i.e.* 45% and 42% for swimmers and non-training girls, respectively (Table 3). According to Nadolna *et al.* [2001], insufficient consumption of milk and dairy products is a highly complex problem. Apart from economic factors, including incomes of population and prices of food products, the preferences of milk and dairy products choice and their intake are also determined by extra-economic factors, including mainly eating habits and nutritional patterns. What is more, the consumers are still little aware of the nutritive value of this group of food products and its significance in the proper development of children and adolescents as well as in health status preservation.

The reduced consumption of milk and dairy products has also been demonstrated in the populations of children from Germany [Libuda *et al*., 2008], China [Du *et al*., 2002] and the USA [Kranz *et al*., 2007].

This study demonstrates a high protein intake from diet both in the case of the swimmers and non-training girls. In both these groups, there were no subjects whose food rations would not cover the estimated average requirement (ERA) for this nutrient (Table 2). Lemon [2001] claims that a higher intake of protein is beneficial in persons training sports. In children and adolescents it is additionally linked with the developmental period. According to Barzel [1995], the high protein intake may, however, disrupt calcium metabolism in the body.

Ample studies have documented the effect of the excessive intake of protein with diet on enhanced calcium excretion with urine [Kerstetter *et al*., 2003; Sellmeyer *et al*., 2001]. Nevertheless, Heaney [2000] is of the opinion that though dietary protein increases calcium excretion with urine obligatorily, its adverse effect on the body occurs only in the case of significant deficiency of this element in diet – below 500 mg/day/person. According to this author, the high intake of protein is strongly correlated with calcium intake, hence protein intake has no significant effect on calcium metabolism in the body [Heaney, 2001, 2002b].

Vatanparast *et al*. [2007] investigated the effect of protein intake on BMC and BMD values in young subjects being in the period of pubescence, with account taken of calcium content in diet. They demonstrated that in the case of insufficient calcium intake, the intake of protein had a positive impact on bone mass of young women.

Heaney [1993] emphasizes the significance of mutual ratios between calcium and protein in a food ration and recommends the calcium to protein ration in diet to account for 16:1 and be considered while evaluating calcium intake. The above ratio of these nutrients assures optimal calcium excretion with urine and the maximum calcium balance in the body. The calcium to protein ratio demonstrated in our study (Table 3) was considerably lesser than the value of 16:1, which – irrespective of age – should be assured by diets of women paying attention to dietary recommendations for protein and calcium.

The alarmingly low intake of calcium with the simultaneously excessive intake of protein and phosphorus as well as improper ratios between calcium and phosphorus and between calcium and protein, observed especially in the case of the non-training girls, might exert a negative effect on osseous tissue mineralization in the period of reaching the peak bone mass.

CONCLUSION

The mean BMC and BMD values did not differentiate the groups compared in the study, however both in the case of female swimmers (19% of the girls) and in the control group (25% of the girls) the study revealed incorrect Z-score values, which points to the need for continued observations in terms of calcium intake (especially amongst the swimmers) and other risk factors of the reduced bone mass.

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