# Influence of dietary calcium intake on quantitative and qualitative parameters of bone tissue in Polish adults

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#### Abstract

**Introduction.** The objective of the study was to assess dietary calcium intake in the Polish population and its influence on selected parameters of bone tissue.

**Materials and method.** 1,129 osteoporosis treatment–naive subjects, aged 20–80 years, randomly selected, were involved in the study. Bone status was established using densitometry of spine and hip and quantitative ultrasound of the calcaneus. Dietary calcium intake was calculated according to data gathered in a questionnaire.

**Results.** Median calcium intake was 746 mg; 72% of subjects had calcium intake below the recommended dose. Calcium intake correlated negatively with age (r = -0.15; p < 0.001) and positively with BMD in the spine (r = 0.06; p < 0.05) and in the femoral neck (r = 0.07; p < 0.05). In subjects with the lowest calcium intake, a significantly lower femoral neck BMD and heel stiffness was noticed than in subjects with the highest calcium intake. However, multiple regression analysis showed that dietary calcium was not a predictor of low BMD, both in the hip and spine, as well as of bone stiffness in contrast to age, low BMI and female gender (p < 0.0001). In all factors regression analysis, a weak influence of calcium intake on BMD was shown only in the subgroup of premenopausal women ( $\beta = 0.1$ ; p < 0.05).

**Conclusions.** In most subjects, dietary calcium intake was below the recommended dose; however, its influence on bone seems to be weak, except for persons with the greatest deficiency of dietary calcium and the subgroup of premenopausal women.

#### Key words

calcium intake, bone mineral density, bone stiffness

## INTRODUCTION

Calcium plays many important roles in the human organism. It is necessary for normal bone structure, neurotransmission, muscle contraction and blood coagulation. Calcium deficiency has a detrimental effect on bone health; low calcium intake increases PTH level and leads to low bone mass and accelerates osteoporosis development [1]. It should be mentioned that dietary calcium intake belongs to modifiable osteoporosis risk factors, in contrast to other,- i.e. genetic factors - which has clinical relevance. A positive effect of dietary calcium on bone mineral density in childhood was shown in the meta-analysis of Wosje and Specker [2]. Also, in post-menopausal women, calcium significantly reduces fracture risk at various skeletal sites [3, 4]. This is why an adequate calcium intake becomes a standard in osteoporosis prophylaxis and treatment. However, many differences among countries exist in recommendations of optimal dietary calcium intake. According to Polish recommendations, adults aged 19-50 years should ingest 1,000 mg of calcium in their everyday diet and those over 50 years old - 1,300 mg [5].

In recent years, several doubts affecting the role of calcium as an osteoporosis risk factor have been raised. In an analysis

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by the US NHANES III base (almost 10,000 subjects) a statistically significant positive association between dietary calcium intake and BMD was observed only in women with low 25(OH)D concentrations, whereas in women with normal 25(OH)D concentrations and in men, calcium intake was not associated with BMD [6]. Some authors did not observe any beneficial effect of calcium intake on fractures risk [7, 8], or this effect was present only in subjects with the biggest calcium deficiency [9]. Currently, calcium intake is not present in any of the fracture risk calculators (e.g. FRAX, Garvan). Moreover, the safety of calcium prophylaxis has been questioned, indicating an increased risk of myocardial infarction [10] and nephrolithiasis development [8].

This cross-sectional study was performed to assess dietary calcium intake in the Polish population and its influence on selected parameters of bone tissue. Quantitative parameters were represented by bone mineral density (BMD), measured by dual-energy X-ray absorptiometry (DXA), while qualitative parameters – by bone stiffness index, measured by ultrasound of calcaneus. Assessment of BMD is a precise, sensitive and reproducible method widely accepted to identify patients at high risk of fractures [11], employed in drug registration studies [12], although BMD is only a surrogate for bone strength. In contrast, ultrasound of the calcaneus is expected to provide information not only about bone density but also on bone quality (mineralization, elasticity and bone structure) [13, 14].

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#### MATERIALS AND METHOD

This research is a part of the EPOLOS study, a Polish multicentre, population-based cross-sectional study on osteoporosis and its risk factors.

Invitations to participate in the study were sent to people aged between 20-80, randomly-selected on the personal identity number basis (PESEL). Participants were enrolled in the following geographic areas: Warsaw, Lodz, Poznan, Crakow and Wroclaw. Exclusion criteria were: personal history of osteoporosis or other bone metabolic diseases, fracture(s) during the previous year, pregnancy, taking any antiosteoporotic drugs or calcium supplements/medications. Participants signed an informed consent to take part in the study. The design of the EPOLOS study was approved by the Ethics Committee of The Children's Memorial Health Institute in Warsaw. Study procedures included measurements of bone mineral density of lumbar spine and femoral neck and stiffness of heel. Calcium intake was estimated with a semi-quantitative method which measured food consumption frequency, based on the intake of calcium in dairy products, according to the data gathered in a food frequency questionnaire (FFQ) [15, 16]. The questionnaire allowed assessment of the dietary calcium intake on the basis of calcium content in 21 different most common types of food and the frequency of eating in Poland. The products included milk (in beverages and soups), different types of cheese (rennet, cottage, cream, processed), yoghurt and ice cream. Calcium contents in individual products was determined by means of a Polish database of nutritional values of food products [17]. Measurements of weight and height were performed and BMI calculated. Finally, all procedures were completed by 1,129 subjects, of whom 677 were women and 452 men.

Densitometry. Lumbar spine and hip BMD were measured by DXA (dual-energy X-ray absorptiometry, using a pencilbeam densitometer DPX or DPX-L(GE Lunar, USA). Scans were performed according to the standard protocol of the operator's manual supplied by the manufacturer. Daily calibration and quality control were performed regularly, according to the manufacturer's instruction. In every centre, two phantoms (anthropomorphic spine phantom and European Spine Phantom) were scanned twice, at the beginning and at the end of the study. There was no need to make any corrections, because neither significant trend in time nor systematic difference between densitometers were detected [18]. All scans were controlled and analyzed by one operator from the central site. Interpretation of densitometry was performed according to ISCD recommendation [19]. The worse result of two localizations (femur, spine) was chosen to establish diagnosis.

**Stiffness index.** Calculated from the values of broadband ultrasound attenuation (BUA) and speed of sound (SOS), which were measured by a heel QUS device (Achilles, Lunar, Co. USA). BUA and SOS measurements employed in this study complied with manufacturer-suggested standards [20]. Daily quality control was performed regularly, according to the manufacturer's instruction. Cross-calibration was carried out on the basis of measurements in volunteers. Results were recalculated for a model device [21].

Statistics. All calculations were made using STATISTICA 10 PL software. The data were presented as mean (± SD) or as median (and quartiles) depending on the distribution of the data. Normality of distribution was tested with Shapiro-Wilk test. Distributed non-parametric variables were tested after Box-Cotrans formation. T-Student or Mann-Whitney tests were used for comparisons between groups. Pearson's or Spearman's coefficients of correlations were calculated. Multiple regression analysis was used to assess an influence of gender, age, calcium intake and BMI on BMD of femoral neck, lumbar spine and stiffness. All factors multivariate linear regression analyses were performed to determine the possible effects of a group of independent variables (age, BMI, calcium intake) on the dependent variables (BMD of spine and femoral neck and stiffness) in subgroups of pre- and postmenopausal women, and men<50 and  $\geq$ 50 years old. The results were considered significant for p<0.05.

#### RESULTS

Finally, 1,129 individuals (452 men, 677 women) were included in the analysis. Characteristics of population shown in Table 1. The analysis was performed in subgroups of preand postmenopausal women, and men <50 and  $\geq$ 50 years of age.

Table 1. Characteristics of study population (median; Q1-Q3)

		Pre- menopausal women N=378	Post- menopausal women N=299	Men <50 yrs N=199	Men≥50 yrs N=253	
Age [yrs]	Median	37.4	61.9	37.5	61.6	
	Q1-Q3	28.3-44.2	55.4–69.8	29.4–45.1	55.6–69.7	
BMI [kg/m²]	Median	23.0	28.1	25.7	27.4	
	Q1-Q3	20.7–26.0	25.0-31.1	23.4–28.1	24.9–29.9	
Spine BMD [g/cm²]	Median	1.178	1.013	1.143	1.126	
	Q1-Q3	1.083–1.268	0.915–1.150	1.084–1.254	1.016-1.261	
Femoral neck BMD [g/cm <sup>2</sup> ]	Median	0.994	0.862	1.050	0.936	
	Q1-Q3	0.910-1.078	0.770-0.948	0.955–1.151	0.852-1.016	
Stiffness [%]	Median	92.6	79.6	98.1	90.2	
	Q1-Q3	84.6-101.7	69.2-90.3	87.0-109.3	81.7–99.6	

BMI - body mass index; BMD - bone mineral density;Q1- first quartile; Q3 - third quartile

**Calcium intake.** In the studied population, the median of calcium intake was 746 mg/daily. Daily dietary calcium intake was below 1,000 mg in 72%; in 70% of investigated women and in 75% of men. The highest median of calcium intake was present in premenopausal women, and it was significantly higher than in postmenopausal women (802 mg/d vs. 725 mg/d; p=0.0055). In the group of men below 50 years of age, calcium intake was higher than in men over 50 yrs (794 mg/d vs. 655 mg/d; p=0.00022) (Fig.1).

In men, the median of calcium intake was lower than in women (709 mg/d vs 770 mg/d; p=0.00895) and in older subjects it was lower than in the those who were younger (700 mg/d vs 798 mg/d; p=0.000003). A negative correlation between calcium intake and age was shown in the general population (r=-0.19; p<0.000001;) as well as in women (r=-0.17; p=0.000005), both premenopausal (r=-0.09; p<0.04)

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Figure 1. Daily calcium intake in studied population.

Bars represent median value; p - level of significance; \*p<0.01 for women vs. men; \*\* p<0.01 for premenopausal women vs. postmenopausal women; \*\*\* p<0.05 for men<50 years vs. men  $\ge$ 50 years

and postmenopausal (r=-0.12; p<0.005). In the group of men, negative correlation between age and calcium intake was shown for all men (r=-0.18; p=0.000075) and subgroup of men <50 years old (r=-0.26; p<0.0005) but not for men >50 years old (r=0.049; p=NS) (Fig. 2).

**BMD.** Most subjects from the younger group had normal BMD: 91% (183/199) in the group of men below 50 years of age, and 95% (361/378) of premenopausal women. In post-menopausal women, 22% (34/299) of subjects were found to have osteoporosis, 51% (135/299) were found to have osteopenia, and 27% (84/299) – normal bone density.

In men over 50 yrs, 13% (67/253) of subjects were found to have osteoporosis, 54% (135/253) – osteopenia and 33% (84/253) – normal bone mass. Distribution of subjects in the studied population according to ISCD classifications is shown in Figure 3 [19]. Mean values of BMD in particular groups is shown in Table 1.

**Stiffness.** The highest median of stiffness index was found in the group of men below 50 years of age, pre-menopausal women, men over 50 and post-menopausal women (Tab. 1).

Influence of calcium intake on BMD/bone stiffness. A weak, but statistically significant correlation between calcium intake and BMD was demonstrated: lumbar spine (r=0.06; p=0.038) and femoral neck (r=0.07; p=0.013). Analogical correlation was present in the subgroups of women and men, but not for age-dependent groups. A similar relationship between calcium intake and stiffness was observed for the whole study group (r=0.06; p=0.026), for both men and for women.

In subjects with the highest calcium intake (over the third quartile) a significantly higher mean femoral neck BMD was noticed than in subjects, whose calcium intake was the lowest, below the first quartile (Fig. 4A). A similar relationship was found for lumbar BMD, but it was not statistically significant for all groups; a statistically significant difference was observed only in women (Fig. 4B).



**Figure 2.** Negative correlation between age and calcium intake.

A - in premenopausal women (r=-0.09; p<0.04); B- in postmenopausal women (r=-0.12; p<0.005); C- in men men <50 years old (r=-0.26; p<0.0005); D - in men >50 years old (r=-0.49; p=NS)

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Figure 3. Distribution of subjects in examined population, according to ISCD classifications [19]



**Figure 4A.** Comparison of femoral neck BMD between subjects with the lowest (Q1 – first quartile) and the highest (Q3 – third quartile) calcium intake. Bars represent mean of BMD in investigated groups; \* level of statistical significance.; \* p < 0.05; \*\* p < 0.01



**Figure 4B.** Comparison of lumbar spine BMD between subjects with the lowest (Q1 – first quartile) and the highest (Q3 – third quartile) calcium intake. Bars represent mean BMD in investigated groups; \* level of statistical significance:

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**Figure 4C.** Comparison of stiffness index between subjects with the lowest (Q1 – first quartile) and highest (Q3 – third quartile) calcium intake. Bars represent mean stiffness in investigated groups; \* p<0.05; \*\* p<0.01; p – level

Bars represent mean stiffness in investigated groups;  $^{\circ}$  p<0.05;  $^{\circ}$  p<0.01; p – level of significance

A statistically significant difference in stiffness between subjects with the highest and lowest calcium intake was shown for the general population (89.01 vs 92.92; p=0.003), as well as for gender-dependent (Fig. 4C).

Multiple regression analysis showed that age was the strongest independent negative factor predicting low BMD both in hip and spine, as well as low bone stiffness (p<0.0001), BMI and female gender were significant positive predictors; however, low calcium in the diet was not found to be a significant factor in the quoted issue. The beta coefficient value for calcium intake was ten times lower than for BMI. Table 2 presents the results of multiple linear regression models fitted to data for lumbar spine and femoral neck BMD and stiffness index. All factors regression analysis showed a weak but significant influence of calcium intake on BMD, but not on stiffness index, limited to subgroup of premenopausal women only. However, the combination of the three predictors (age, calcium intake and BMI) accounted for nearly 7–15% of BMD. In the other groups, the influence of calcium intake on BMD and stiffness index was not statistically significant in contrast to effect of age and BMI (Tab. 3).

 Table 2. Multiple regression analysis results: influence of gender, age, calcium intake and BMI on BMD of femoral neck, lumbar spine and stiffness

	Femoral Neck BMD (overall r=0.58; p<0.0001)		<b>Lumbar</b> <b>BMD</b> (overall r=0.35; p<0.0001)		Stiffness (overall r=0.51; p<0.0001)	
	BETA	Р	BETA	р	BETA	р
Gender [female]	-0.19	p<0.0001	-0.12	p<0.0001	-0.23	p<0.0001
Age [years]	-0.61	p<0.0001	-0.35	p<0.0001	-0.51	p<0.0001
Calcium intake [mg/daily]	0.03	p=0.30	0.03	p=0.24	0.02	0.35
3MI [kg/m²]	0.33	p<0.0001	0.25	p<0.0001	0.21	p<0.0001

BMI – body mass index; BMD – bone mineral density; BETA – regression coefficient value; r –correlation coefficient value; p –level of significance

### DISCUSSION

The EPOLOS study confirmed dietary calcium deficiency in the Polish population. Median calcium intake was 770 mg/d in women and 709 mg/d in men, and more than 72% of subjects had calcium intake lower than 1,000 mg/d, which is the average daily intake recommended in the Polish adult population. The problem of low calcium intake is widespread and has been observed in the United States [22] and many European countries [23, 24]. The situation was similar in the Polish population, as previously shown [25, 26, 27]. In the presented study, calcium intake was found to be higher than in other studies in which this amount was about 600-700 mg/d. The lowest intake was shown in the RACPOL study: 336 mg/daily in women with previous fractures and 395 mg/daily in women without fractures [27]. This discrepancy may result from population differences: females involved in the RACPOL were older, they came from one small region, and finally, a history of osteoporosis was not a study exclusion criterion. In contrast, subjects in the EPOLOS study comprised a bigger population, which included both men and women, recruited from treatment

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Table 3. All factors analysis results; beta-standarized regression coefficient

	Lumbar BMD			Femoral neck BMD			Stiffness		
	β	р	R2 (p)	β	р	R2 (p)	β	р	R2 (p)
Pre-menopausal women									
Age	-0.11	<0.05	0.07	-0.25	<0.001	_ 0.147 _ (p<0.000)	-0.36	<0.00	0.112 (p<0.000)
Ca intake	0.10	<0.05	(p<0.000)	0.09	<0.05		0.03	NS	
BMI	0.29	<0.001		0.4	<0.001		0.24	<0.000	
				Post-menopa	usal women				
Age	-0.19	<0.001	0.138	-0.42	<0.001	0.278	-0.41	<0.001	0.236 (p<0.000)
Ca intake	0.001	NS	(p<0.000)	-0.01	NS	(p<0.000)	-0.03	NS	
BMI	0.33	<0.001		0.34	<0.001		0.28	<0.001	
				Men <50	) years				
Age	-0.06	NS	0.016	-0.33	<0.000	0.178	-0.19	< 0.05	0.040 (p<0.01)
Ca intake	0.02	NS	(p=NS)	0.12	NS	– (p<0.000) –	0.06	NS	
BMI	0.18	< 0.05		0.37	<0.001		0.17	< 0.05	
				Men≥50	years				
Age	0.07	NS	. 0.077 . (p<0.000)	-0.21	<0.001	0.152	-0.13	0.21	0.057 (p<0.001)
Ca intake	-0.01	NS		-0.01	NS	(p<0.000)	0.01	NS	
BMI	0.29	<0.001		0.32	<0.001		0.21	<0.01	

R2 – coefficient of determination; p – level of significance; BMI – body mass index; BMD – bone mineral density R2 – coefficient of determination; p – level of significance; BMI – body mass index; BMD – bone mineral density

naive individuals from several centres, while their age range was much broader (18–80 yrs).

The current study shows that calcium intake depends on age. The lowest calcium supply was observed in the oldest age group. This deficit could be regarded as serious, because in that age group the recommended dose of calcium should be the highest. The presented findings about the relationship between age and calcium intake are supported by others [6, 28]. A bigger consumption of calcium declared by younger individuals may result from greater awareness of osteoporosis risk among younger patients [29].

The results of this study demonstrate that calcium supply was also gender-dependent: it was found that women consume significantly more calcium than men (770 mg/d vs. 709 mg/d). Lower calcium intake by men seems to be related to dietary habits of Polish men, since it was noted also by Ilow in the PONS study, a prospective investigation evaluating mineral and vitamin intake in the Polish population [30], but not in studies from other countries [6, 30].

In order to assess the clinical significance of dietary calcium deficiency this study investigated the relationship between calcium intake and BMD, as well as bone stiffness. According to the data obtained, the influence of calcium on bone parameters is not strong and may depend on the type of the bone (cortical or trabecular). A weak, but statistically significant correlation was found between calcium consumption and BMD, more strongly expressed in the cortical bone (measured in the femoral neck) than in the trabecular bone (measured in the lumbar spine). However, multiple regression analysis demonstrated that calcium intake is not a significant predictor of BMD in contrast to advanced age, low BMI and female gender. The obtained data are consistent with the study of Bischoff-Ferrari et al. [6], who found no significant association between a higher calcium intake and BMD, except for women with low vitamin D concentrations. Both in the report by Bischoff-Ferrari et al. [6] and the current study, calcium came from diet only; subjects who supplemented calcium were excluded and the scope of age was wide. Stepwise analysis showed that the influence of calcium intake on BMD is statistically significant, but weak and limited only to the group of premenopausal women. That results reflect differences between the male and female pattern of bone growth, and suggest that a higher calcium intake is able to increase peak bone mass improving bone status in young women, supporting the thesis about benefits from prophylaxis with calcium-rich products in that group of patients.

Analysing the relationship between calcium intake and stiffness index, a weak positive correlation was found which, however, was not significant in multiple regression and in all factors regression analysis in all studied groups.

The current data affecting the influence of calcium supply on bone stiffness are discrepant; low calcium supply was shown to be a predictor of bone stiffness in premenopausal women [31] and adolescent girls [32]. However, in Japanese women, stiffness was not related to dietary calcium intake [33], while an analysis of postmenopausal Italian nuns showed that vitamin D deficiency is more important in the reduction of stiffness than high calcium intake [34].

The current study demonstrates a significant difference in BMD and stiffness between subjects with the lowest and the highest calcium dietary intake. A similar observation was made by Warensjö [9], who noticed an increased risk of osteoporosis in the lowest quintile of dietary calcium intake. In the KNHANES study, BMD in the lumbar spine and femoral neck was significantly lower only when calcium intake was less than 400 mg/d [35]. In the light of the obtained data, it appears that the prophylactic strategy should include, in particular, the identification of persons with the greatest deficiency of dietary calcium, and the implementation of prevention strategy in this particular group.

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The limitations of the presented study include the lack of vitamin D concentration data. This is a crucial point, since vitamin D enhances the renal conservation and intestinal absorption of calcium, and the Polish population is vitamin D deficient [36]. The design of the study, however, reflects the standard clinical practice in Poland during the period of data collection, i.e. when routine measurements of vitamin D concentrations were not performed in clinical settings. Using BMD as the endpoint rather than a fracture may underestimate the effect of calcium, as observed in clinical trials of antiresorptive drugs, where reduction of fracture risk was higher than would be predicted based on their effect on bone density [12]. On the other hand, BMD seems to be more reliable in premenopausal women, in which fracture incidence is low. Another potential factor interacting with calcium and BMD, not included in this study, is physical activity: meta-analysis of 17 studies demonstrated that the positive effect of calcium appears more likely in those who exercised than in those who did not [37].

Despite the drawbacks of the study, its strengths should also be emphasized, which include a wide range of subject population through the system of random, multicentre recruitment - one of the biggest Polish population samples, and the prospective design of the study. In this way, a population of individuals was recruited who, prior to the study, had considered themselves to be entirely healthy. In this model, it was possible to eliminate the reverse causation phenomenon, occurring in people with osteoporosis, who under the influence of its recognition change their eating habits by introducing calcium rich products into their diet. Therefore, the design of the study reflected what might be encountered if population-based preventive measures are undertaken, as it was directed towards a presumably healthy (and treatment-naive) population of individuals, rather than to patients already attending osteoporosis clinics.

#### CONCLUSIONS

Among the examined Polish adults, dietary calcium intake was below the recommended dose; however, its influence on bone quantity and quality seems to be weak, except for persons with the greatest deficiency of dietary calcium and the group of premenopausal women.

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