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Bone mineral density in the various regions of the skeleton in women with subclinical hypothyroidism: the effect of biological factors, bone turnover markers and physical activity

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Abstract

Study aim: This cross-sectional study examined the relationship between biological factors, physical activity (PA), bone turnover markers (BTMs) and bone mineral density (BMD) in women with subclinical hypothyroidism (SCH) and healthy.

Material and methods: The study included 135 women. Bone parameters were measured by the densitometry. Calcium, phosphorus, osteocalcin (OC), total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), vitamin D and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) were measured in blood serum. PA were evaluated by metabolic equivalent of task (MET).

Results: Women with SCH had lower BMD in hip and lumbar spine, calcium, vitamin D and higher values of ICTP than the healthy group. In women with SCH, the affecting BMD in the femoral neck were ICTP (μg/l), ($\eta^2 = 0.083$), and also 25(OH)D (ng/ml), ($\eta^2 = 0.080$) and PA level (I/S), ($\eta^2 = 0.115$). BMD in the spine was affected by PA level (I/S), ($\eta^2 = 0.173$). The parameters affecting BMC in the femoral neck were 25(OH)D (ng/ml), ($\eta^2 = 0.073$). In all women sufficient levels of PA determined higher BMD.

Conclusions: PA and BTMs significantly determine BMD levels. PA can be an important area of rehabilitation and physical therapy as an available measure to counteract BMD loss in postmenopausal healthy women and those with SCH.

Keywords: Hypothyroidism – Bone mineralization – Bone formation and resorption biomarkers – Physical activity

Introduction

Bone remodeling is a continuous process characterized by alternating resorption and bone formation phases. The sequence of events in remodeling includes the stages of activation, resorption, and bone formation. The final product of the remodeling process is a bone tissue structure with a specific structure and mechanical strength [11, 8]. Bone mineral homeostasis is predominantly controlled by parathyroid hormone (PTH), calcitonin, and the active form of vitamin D. These factors play an important role in maintaining mineral homeostasis and normal development of bone tissue [18]. Vitamin D3 is metabolized in the liver by 25-hydroxylase enzyme. As a result of this process, 25 hydroxyvitamin D [25(OH)D] is produced. In the next stage, it is transformed by the kidneys into an active form of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by 1-α hydroxylase enzyme. Important for bone health are serum 25(OH)D and 1,25$(OH)_2$D as they are indicators of nutritional status, stimulate calcium absorption, and regulate bone turnover. Activity of 1,25$(OH)_2$D leads to an increase in the concentration of serum calcium while decreasing PTH. PTH secretion from the parathyroid glands regulates serum calcium and 1,25$(OH)_2$D levels. PTH stimulates bone turnover. Bone mineral homeostasis is closely determined by the levels of concentrations of dependent biomarkers [8, 23].

Bone turnover markers (BTMs) can be used to monitor bone metabolism. Biochemical markers for assessing bone turnover are primarily enzymes and non-enzymatic peptides. Based on the metabolic phase during which

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they are produced, BTMs are grouped into two categories: bone formation markers and bone resorption markers [25, 31]. Markers commonly used as bone formation markers include matrix proteins – osteocalcin (OC), by-products of collagen synthesis such as propeptides of type I (C-terminal: P1CP, N-terminal: P1NP), and osteoblast enzymes such as serum bone-specific alkaline phosphatase (BALP). In many epidemiological and clinical studies as well as in the diagnosis of bone conditions, OC is the most commonly used bone formation marker. On the other hand, the most common markers used to assess the rate of bone resorption are serum C-terminal telopeptide of type I collagen (ICTP). ICTP is a marker of increased bone resorption and it is formed during the degradation of collagen fibers [34]. The combination of BMD measurement by dual-energy X-ray absorptiometry (DXA) and BTMs detection shows great potential to improve the early assessment of people with a high risk of osteoporosis [23]. General health and various diseases can also affect BMD. Studies indicate that thyroid disease can significantly affect BMD in different populations [6, 30].

Thyroid hormones play an important physiological role in maintaining bone structure and strength. They are also considered essential elements of the normal skeletal structure. It is known from clinical studies that thyroid dysfunction increases the risk of bone diseases such as osteoporosis. However, the role of thyroid hormone excess or deficiency in the pathogenesis of osteoporosis and risk factors for low BMD needs further research to understand the mechanisms of these interactions [3, 6]. As indicated by several studies, hypothyroidism can slow down bone remodeling processes. Disorders of hormone production and reduction in thyroid hormone levels can gradually lead to disorders in the processes of bone cell formation, maturation, and activity. However, scientific results are inconsistent [6]. Assessment of the relationship between subclinical thyroid dysfunction and change in bone mineral density in prospective cohorts showed that subclinical hypothyroidism was not associated with increased bone loss at any body site [30]. The results of five population-based cohort studies suggest that subclinical hypothyroidism causes an increased risk of osteoporotic fractures in a population of people aged 60 years or older. However, the nature of the association between subclinical hypothyroidism and low BMD and fracture risk has not been fully defined [38]. A review of studies on the association of BMD and the risk of fractures in hypothyroidism shows that the available data are still insufficient and need to be supplemented [12].

The current gold standard test for the diagnosis of bone health is BMD assessment using DXA scanning. BMD level is determined by factors such as gender, age, body weight, body composition, family fracture history, and lifestyles (smoking, alcohol addiction, physical activity, and nutritional habits) [16, 20, 21]. Studies suggest that physical activity, especially strength training using weights and resistance exercises positively affects the bone metabolism of women in different age groups. The beneficial effect of physical activity on bone health results from the axial load. Adequate levels of physical activity in childhood and adolescence when peak bone mass is observed are very important. If continued in later stages of life, it is an optimal solution to ensure bone health [21, 37]. The associations of physical activity with BMD in women with hypothyroidism have not been sufficiently studied. In this study, we hypothesized that physical activity may be a protective factor for BMD in hypothyroid women as well as in healthy women.

This study examined the relationship between biological factors such as age at menarche, age at menopause, body mass index (BMI), and also physical activity (PA), bone turnover markers (BTMs) and bone mineral density (BMD) in the various regions of the skeleton in women with subclinical hypothyroidism (SCH) and healthy.

Material and methods

Participants and procedures

This cross-sectional study involved 135 postmenopausal women aged 57.4 ± 6.3 years of the same ethnic origin (Caucasians of European origin), including 68 patients with subclinical hypothyroidism (SCH) and 67 healthy female controls matched for age. All participants gave written consent to participationn on reserch. The general characteristics of the study group are shown in Table 1.

The project is in compliance with the principles of bioethics, as confirmed by the Bioethics Committee of the National Institute of Public Health, National Institute of Hygiene in Warsaw (protocol number 1/2021). The work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All individual participants provided informed.

The study sample comes from a large longitudinal study "Determinants of Bone Density of the Polish Population 2020-2030". The study was conducted from September 2022 to March 2023 in the densitometry and anthropometry laboratory in the Anthropology Section, Department of Human Biology. The study included women living in a large metropolitan area. Recruitment and inclusion criteria: Caucasian women of European descent, volunteers applying from the announcement of the study, age group from 40 to 60 years, no health contraindications to the study, written consent to participate in the study. Next, using the purposive selection method, the results of women with subclinical hypothyroidism were included in the analyses. Diagnosis of subclinical hypothyroidism

Table 1. The biometric and somatic characteristics women with SCH and healthy

Variables	Women with SCH (n = 68)	Healthy women (n = 67)	t (p)	Hegdes' g
	Mean ± SD	Mean ± SD		
Biological parameters				
Age [year]	58.0 ± 6.7	56.8 ± 5.7	1.107 (0.270)	0.194
Age at menarche [year]	12.9 ± 1.6	12.8 ± 1.84	0.181 (0.856)	0.058
Age at menopause [year]	50.3 ± 3.21	49.9 ± 2.84	0.692 (0.490)	0.132
Body Height [cm]	163.4 ± 6.19	164.7 ± 5.54	–1.301 (0.196)	0.222
Body Weight [kg]	70.7 ± 16.4	69.1 ± 11.8	0.642 (0.522)	0.113
BMI [kg/m^2]	26.4 ± 5.7	25.5 ± 4.4	1.071 (0.286)	0.178

SCH was conducted by an endocrinologist and confirmed by the result of laboratory tests. Women were under the care of an endocrinology clinic. Thyrotropin and free thyroxine levels were measured from serum samples in Roche Elecsys 2010 Analyzer (Roche Diagnostics Corp). In this study used a immunoassay method and competition immunoassay method, respectively. Subclinical hypothyroidism was defined as a thyrotropin level higher than 5.1 mIU/L, in the setting of a normal free thyroxine level ranging from 0.85 to 1.4 ng/dL.

The entire research project was carried out in certified laboratories. A uniform test procedure was used for all project participants, the same equipment, daily calibration procedures and measurement methodology. All women in the study reported their hormonal status in face-to-face interviews. Furthermore, the collected data was compiled according to the hormonal status classification recommended by the World Health Organization (WHO) [29]. Postmenopausal women were included in the study.

The exclusion criteria were bone diseases, rheumatoid arthritis, kidney disease, hormone replacement therapy, cancers, nutritional disorders, and long-term steroid treatment. Women who gave written consent to participate in the study were qualified for all stages of the study.

Bone tissue parameters and anthropometric measurements

All bone parameters such as bone mineral density (BMD), bone mineral content (BMC), and T-scores of the lumbar spine and nondominant hip were measured by the densitometry method (dual-energy X-ray absorptiometry, DXA). All bone measurements were taken on a Norland XR-46 bone densitometer (Swissray-USA, Norland Medical Systems Madison WI, USA). The analyses used the T-score calculated using the following formula: patient's BMD – young normal mean/SD of young normal according to the recommendations of a WHO. Data analysis was performed in accordance with WHO definitions of osteoporosis for T-score ≤ -2.5 [9]. This study used the various regions of interest (ROIs): the lumbar spine L1-L4 (LS) and the proximal femur (femoral neck, total hip). The precision error as a percentage coefficient of variance was less than 1% during the study period. DXA scans (total and subregional) were obtained for all participants included in the present study and were performed by certified radiology technicians. According to the densitometric testing procedure and recommendations of the International Society for Clinical Densitometry, the scanner was calibrated daily. The calibration was performed against the standard calibration block supplied by the manufacturer to control for possible baseline drift.

Somatic measurements such as body height and body weight were also taken, and the body mass index (BMI) was used. Anthropometric measurements were performed according to standard methods by an anthropologist [15].

Biochemical markers of bone turnover

The serum biomarker concentrations were measured as recommended by the Joint Committee on Bone Metabolism of the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [7].

Concentration levels were marked according to medical procedures. Blood samples were taken from the elbow vein at rest in the morning on an empty stomach. Vitamin D and its two active metabolites were assessed based on serum 25-hydroxyvitamin D [25(OH)D] and 1,25 – dihydroxyvitamin D [1,25$(OH)_2$D] concentrations (in ng/mL). Biochemical determinations were made using the method of chemiluminescent immunoassay (CLIA) using the IDS-iSYS analyzer certified by the Vitamin D External Quality Assessment Scheme (DEQAS). Calcium (mmol/L) and phosphorus (mmol/L) levels were also assessed based on their serum concentrations. Serum levels of bone biomarkers measured were osteocalcin (OC) (Bone Gla Protein), total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP) as markers of bone formation, and cross-linked carboxyterminal telopeptide of type

I collagen (ICTP) as a marker of bone resorption. Concentrations were determined by the enzyme-linked immunosorbent assay (ELISA) human kit based on the principle of competitive binding. ICTP concentrations were measured using the radioimmunoassay method (RIA). The serum osteocalcin (OC) levels were evaluated by Elecsys N-MIND Osteocalcin test (Roche) using the ECLIA method on a Cobas apparatus.

Physical activity evaluation method

The International Physical Activity Questionnaire (IPAQ, short version) was used to evaluate levels of physical activity. IPAQ is recommended in such international studies as the European Health Interview Survey (EUROHIS) and the European Physical Activity Surveyance System (EUPASS). The metabolic equivalent of task (MET) was used in the analysis. Weekly physical activity was calculated by adding up MET values obtained during vigorous activity, moderate activity, and walking performed over the entire week. Two levels of weekly physical activity were recorded (insufficient and sufficient). Physical activity levels were classified as insufficient for MET-min/week ≤600, whereas sufficient physical activity – for MET min/week >600 [24].

Statistical analysis

The research results were analysed with the use of Statistica software (v.11, Stat. Soft., USA). In order to determine the significance of differences between the values of particular variables for women with SCH and healthy women, Student's t test for independent variables was applied. In order to investigate the nature of the distribution of the results of the Shapiro-Wilk test was conducted. In case of rejecting the assumptions of normality of the assessment distribution of the significance of diversity we used the Kruskal-Wallis test. The size of the difference between the results of the group with SCH and the healthy group was calculated using the 'Hedges G' formula (small effect: <0.5; medium effect: 0.5–0.8; large effect: >0.8). Differences between the frequency of occurrence of appropriate and decreased bone mineralization in femoral neck and lumbar spine were analysed with the use of the chi-square test. In order to find relationships between BMD, BMC and BMI, variables of biological age, bone turnover parameters and physical activity (PA) the ANCOVA was applied (PA as qualitative predictor). The degree of correlation of the predictors was assessed using the variance inflation factor (VIF) collinearity test, taking a not-to-exceed value of 10. Residual analysis was also performed, testing for homoscedasticity using the White test and the degree of correlation of the residuals using the Durbin-Watson test. Effect size was calculated as eta-squared (η^2) (small effect: <0.06; medium effect: 0.06–0.14; large effect: >0.14). Two-way ANOVA analysis was applied to determine the relationships of selected bone parameters (BMD, BMC), physical activity levels (insufficient and sufficient) and women's health status (with SCH and healthy). Statistical significance level was set at $\alpha = 0.05$.

Results

The comparison of the bones parameters, bone turnover markers and physical activity in women with SCH and healthy are presented in Table 2. The groups differed significantly in 11 of the 18 analyzed parameters. Women with SCH had statistically significantly lower bone parameters: femoral neck BMD (large effect >0.8) and femoral neck T-score, total hip BMD, total hip T-score, lumbar spine BMD, and lumbar spine T-score (medium effects at 0.5–0.8). Analogous calculations were made for bone turnover markers. Significantly higher values of ICTP (μg/l) as a marker of bone resorption were noted in women with SCH than in the healthy group (medium effect: 0.5–0.8). In women with SCH, significantly lower values of calcium (mmol/l) (medium effect: 0.5–0.8), $1,25(OH)_2D$ (pg/ml), and 25(OH)D (ng/ml) were found compared to healthy women (small effect: <0.5). Women with SCH had significantly lower physical activity by 206.8 MET min./week (small effect size: <0.5) than healthy women (Table 2).

Table 3 presents the frequency of occurrence of normal and decreased bone mineral status in three ROIs: femoral neck, total hip, and lumbar spine in women with SCH and healthy women. The desired value (T-score – 1.0 or above) of femoral neck BMD occurred significantly more often in healthy women (by 22.7%), while in the lumbar spine, it was more frequent by 6.5%. Significant differences ($p < 0.01$) in the incidence of low BMD and osteoporosis were found for the femoral neck between patients and healthy women. Low BMD characteristics of osteopenia occurred significantly more often in women with SCH (by 11.1%). Furthermore, the incidence of osteoporosis in the femoral neck was significantly higher (by 11.6%) in women with SCH (Table 3).

The results of the covariance analyses between BMD and selected parameters indicated that in the case of women with SCH, the main parameters affecting BMD in the femoral neck were ICTP (μg/l), (medium effect: $\eta^2 = 0.083$), and also 25(OH)D (ng/ml), (medium effect: $\eta^2 = 0.080$) and PA level (I/S), (medium effect: $\eta^2 = 0.115$). BMD in the lumbar spine was affected by PA level (I/S), (large effect: $\eta^2 = 0.173$). Similar analyses were conducted for BMC. The results of the covariance analyses between BMC and selected parameters indicated that in the case of women with SCH, the main parameter affecting BMC in the femoral neck were 25(OH)D (ng/ml), (medium effect: $\eta^2 = 0.073$), (Table 4a).

Table 2. Comparison of the bones parameters, bone turnover markers and physical activity in women with SCH and healthy

Variables	Women with SCH (n = 68)	Healthy women (n= 67)	t (p)	Hegdes' g
	Mean ± SD	Mean ± SD		
Bone parameters				
BMD Femoral neck [g/cm^2]	0.719 ± 0.080***	0.833 ± 0.164	–5.142 (<0.001)	0.934
BMC Femoral neck [g]	3.958 ± 0.686	4.166 ± 0.716	–1.717 (0.088)	0.297
T-score Femoral neck	–1.555 ± 0.806**	–1.037 ± 1.305	–2.778 (0.006)	0.491
BMD Total Hip [g/cm^2]	0.836 ± 0.126***	0.916 ± 0.155	–3.274 (0.001)	0.569
BMC Total Hip [g]	29.271 ± 5.177	30.768 ± 5.322	–1.657 (0.100)	0.285
T-score Total Hip	–0.713 ± 0.937**	–0.229 ± 1.225	–2.576 (0.011)	0.448
BMD Lumbar spine [g/cm^2]	0.956 ± 0.245***	1.103 ± 0.232	–3.569 (<0.001)	0.616
BMC Lumbar spine [g]	60.077 ± 10.122	63.419 ± 14.280	–1.570 (0.119)	0.274
T-score Lumbar spine	–0.818 ± 1.135***	–0.088 ± 1.286	–3.500 (0.001)	0.603
Biochemical parameters				
ICTP [µg/l]	5.76 ± 1.02***	5.06 ± 0.92	4.215 (<0.001)	0.722
OC [ng/ml]	19.29 ± 8.11	21.06 ± 8.90	–1.205 (0.231)	0.208
ALP [U/l]	76.63 ± 24.23	71.73 ± 19.55	1.291 (0.199)	0.224
BALP [% of ALP]	41.68 ± 5.52	41.15 ± 4.79	0.597 (0.551)	0.103
Calcium [mmol/l]	2.33 ± 0.08**	2.39 ± 0.12	–3.221 (0.002)	0.600
Phosphorus [mmol/l]	1.15 ± 0.15	1.11 ± 0.14	1.763 (0.080)	0.133
1,25[OH]$_2$D [pg/ml]	49.31 ± 18.90*	56.54 ± 18.42	–2.122 (0.026)	0.387
25[OH]D [ng/ml]	21.85 ± 7.81*	25.15 ± 9.49	–2.104 (0.029)	0.382
Physical activity parameter				
MET [min/week]	651.2 ± 482.2*	858.0 ± 593.1	–2.224 (0.028)	0.385

BMD – bone mineral density; BMC – bone mineral content; OC – osteocalcin; ALP-total alkaline phosphatase; BALP – bone-specific alkaline phosphatase; ICTP – Cross-linked Carboxyterminal Telopeptide of Type I Collagen; Significantly different than Healthy: *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001.

Table 3. Frequency of osteopenia and osteoporosis by T-score in three ROIs: femoral neck, total hip and lumbar spine

Variables/Classification	Women with SCH (n = 68)	Healthy women (n = 67)	Chi-square test (p)
	n		
Femoral neck T-Score			
Normal	15 (22.1%)**	30 (44.8%)	9.107 (0.009)
Low BMD/Osteopenia	39 (57.4%)**	31 (46.3%)	
Osteoporosis	14 (20.5%)**	6 (8.9%)	
Total hip T-Score			
Normal	44 (64.7%)	46 (68.7%)	3.894 (0.079)
Low BMD/Osteopenia	24 (35.3%)	18 (26.9%)	
Osteoporosis	0 (0.0%)	3 (4.5%)	
Lumbar spine T-Score			
Normal	23 (33.8%)	27 (40.3%)	0.841 (0.657)
Low BMD/Osteopenia	43 (63.2%)	39 (58.2%)	
Osteoporosis	2 (3.0%)	1 (1.5%)	

Classification BMD: Normal when T-score – 1.0 or above; Low BMD/Osteopenia when T-score between – 2.5 and – 1.0; Osteoporosis when T-score of – 2.5 or below; Significantly different than Healthy: *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001.

Table 4a. Relationships between BMD, BMC in two ROIs: femoral neck and lumbar spine and BMI, variables of biological age, bone turnover parameters and physical activity levels (MET) in women with subclinical hypothyroidism (SCH) (results of ANCOVA, F test, p)

	BMD Femoral neck (g/cm^2)	BMD Lumbar spine (g/cm^2)	BMC Femoral neck (g)	BMC Lumbar spine (g)
	F (p) η^2	F (p) η^2	F (p) η^2	F (p) η^2
BMI [kg/m2]	0.057 (0.812) 0.001	1.375 (0.246) 0.024	0.001 (0.972) 0.000	0.534 (0.468) 0.010
Age at menarche [year]	1.684 (0.200) 0.030	1.012 (0.319) 0.018	0.009 (0.923) 0.000	4.538 (0.038) 0.076
Age at menopause [year]	0.091 (0.764) 0.002	0.044 (0.835) 0.001	0.127 (0.723) 0.002	0.008 (0.931) 0.000
ICTP [µg/l]	4.948 (0.030)* 0.083	0.023 (0.879) 0.000	0.103 (0.750) 0.002	0.131 (0.719) 0.002
OC [ng/ml]	0.118 (0.736) 0.002	0.000 (0.993) 0.000	0.519 (0.474) 0.009	0.023 (0.880) 0.000
ALP [U/l]	0.294 (0.590) 0.005	0.227 (0.636) 0.004	2.225 (0.142) 0.039	1.162 (0.286) 0.021
BALP [% of ALP]	1.109 (0.297) 0.020	0.109 (0.743) 0.002	0.973 (0.328) 0.017	0.856 (0.359) 0.015
Calcium [mmol/l]	2.915 (0.093) 0.050	0.894 (0.349) 0.016	3.641 (0.062) 0.062	2.183 (0.145) 0.038
Phosphorus [mmol/l]	3.260 (0.076) 0.056	0.409 (0.525) 0.007	0.410 (0.525) 0.007	0.156 (0.694) 0.003
1,25[OH]$_2$D [pg/ml]	0.619 (0.435) 0.011	3.336 (0.073) 0.057	0.080 (0.779) 0.001	3.635 (0.062) 0.062
25[OH]D [ng/ml]	4.813 (0.032)* 0.080	0.052 (0.821) 0.001	4.324 (0.042)* 0.073	0.605 (0.440) 0.011
PA level [I/S]	7.135 (0.010)** 0.115	11.476 (0.001)*** 0.173	3.151 (0.081) 0.054	3.581 (0.064) 0.061

BMD – bone mineral density; BMC – bone mineral content; OC – osteocalcin; ALP-total alkaline phosphatase; BALP – bone-specific alkaline phosphatase; ICTP – Cross-linked Carboxyterminal Telopeptide of Type I Collagen; PA level (I/S) – physical activity insufficient and sufficient; The levels of statistical significance: *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$.

The results of the covariance analyses between BMD and selected parameters revealed that in the case of healthy women, the main parameter affecting BMD in the femoral neck was PA level (I/S),(large effect: $\eta^2 = 0.170$). Furthermore, BMD in the lumbar spine was affected by Phosphorus (mmol/l), (medium effect: $\eta^2 = 0.089$) and PA level (I/S), (medium effect: $\eta^2 = 0.096$). The results of the covariance analyses between BMC and selected parameters revealed that in the case of healthy women, the main parameter affecting BMC in the femoral neck and also BMC in the lumbar spine was affected only PA level (I/S), (large effects: $\eta^2 = 0.214$ and $\eta^2 = 0.281$), (Table 4b).

Figure 1 presents the relationships between femoral neck BMD, physical activity levels, and health status (two-way ANOVA results) of the women studied. There were clear significant interactions of the variables studied. Healthy women with a sufficient level of physical activity had the most advantageous values of femoral neck BMD. In both groups of women (with SCH and healthy participants), sufficient levels of physical activity significantly determined higher BMD (Fig. 1).

Table 4b. Relationships between BMD, BMC in two ROIs: femoral neck and lumbar spine and BMI, variables of biological age, bone turnover parameters and physical activity levels (MET) in healthy women (results of ANCOVA, F test, p)

	BMD Femoral neck (g/cm^2)	BMD Lumbar spine (g/cm^2)	BMC Femoral neck (g)	BMC Lumbar spine (g)
	F (p) η^2	F (p) η^2	F (p) η^2	F (p) η^2
BMI [kg/m2]	2.728 (0.104) 0.048	0.567 (0.455) 0.010	0.126 (0.724) 0.002	0.055 (0.816) 0.001
Age at menarche [year]	0.075 (0.785) 0.001	2.398 (0.127) 0.043	0.497 (0.484) 0.009	1.439 (0.236) 0.026
Age at menopause [year]	0.220 (0.641) 0.004	0.309 (0.580) 0.006	0.015 (0.904) 0.000	3.673 (0.061) 0.064
ICTP [µg/l]	0.383 (0.539) 0.007	1.017 (0.318) 0.018	1.668 (0.202) 0.030	0.017 (0.895) 0.000
OC [ng/ml]	1.141 (0.290) 0.021	2.101 (0.153) 0.037	0.060 (0.808) 0.001	0.752 (0.390) 0.014
ALP [U/l]	1.315 (0.257) 0.024	1.083 (0.303) 0.020	0.238 (0.627) 0.004	0.465 (0.498) 0.009
BALP [% of ALP]	0.062 (0.805) 0.001	0.802 (0.374) 0.015	0.191 (0.664) 0.004	1.475 (0.230) 0.027
Calcium [mmol/l]	3.176 (0.080) 0.056	0.618 (0.435) 0.011	0.905 (0.346) 0.016	0.149 (0.701) 0.003
Phosphorus [mmol/l]	0.518 (0.475) 0.009	5.300 (0.025)* 0.089	0.001 (0.970) 0.000	3.290 (0.075) 0.057
1,25[OH]$_2$D [pg/ml]	1.343 (0.252) 0.024	1.280 (0.263) 0.023	0.642 (0.427) 0.012	0.217 (0.643) 0.004
25[OH]D [ng/ml]	0.615 (0.436) 0.011	1.777 (0.188) 0.032	0.017 (0.897) 0.000	3.823 (0.056) 0.066
PA level [I/S]	11.09 (0.002)** 0.170	5.725 (0.020)* 0.096	14.70 (0.000)*** 0.214	21.08 (0.000)*** 0.281

BMD – bone mineral density; BMC – bone mineral content; OC – osteocalcin; ALP-total alkaline phosphatase; BALP – bone-specific alkaline phosphatase; ICTP – Cross-linked Carboxyterminal Telopeptide of Type I Collagen; PA level (I/S) – physical activity insufficient and sufficient; The levels of statistical significance: *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$.

Figure 2 presents the relationships between lumbar spine BMC, physical activity levels, and health status (two-way ANOVA results) of the women studied. In both groups of women (with SCH and healthy participants), sufficient levels of physical activity significantly determined higher lumbar spine BMC (Fig. 2).

Discussion

Thyroid hormones are essential for the normal formation of bone tissue and maintaining adequate bone strength in adults. Studies have suggested that thyroid dysfunction represents a risk factor for osteoporosis. However, the role of thyroid hormone excess or its deficiency in the pathogenesis of bone diseases has not been fully investigated [12]. Some studies indicate that hyperthyroidism is a risk factor for decreased bone mineral density (BMD) and fractures [14, 36] whereas some found that it was not, except during thyroxine over-replacement [1]. Vestergaard et al. [35] concluded that bone fracture risk in patients with hypothyroidism is increased. In a study of 60 years old patients with SCI, these researchers showed increased fracture risk with a peak around the time of diagnosis

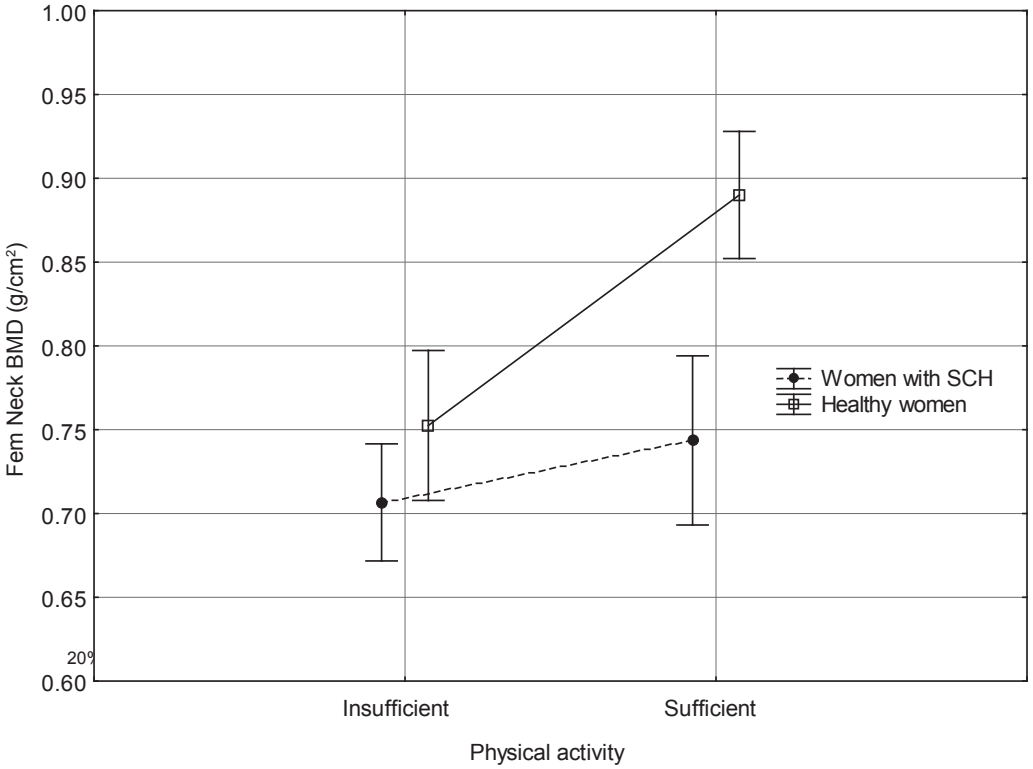


Figure 1. Relationships of BMD femoral neck, physical activity levels and women's health status (two-way ANOVA results, $F_{1, 131} = 5.4892$, $p = 0.021$), vertical lines – 0.95 CI – confidence intervals

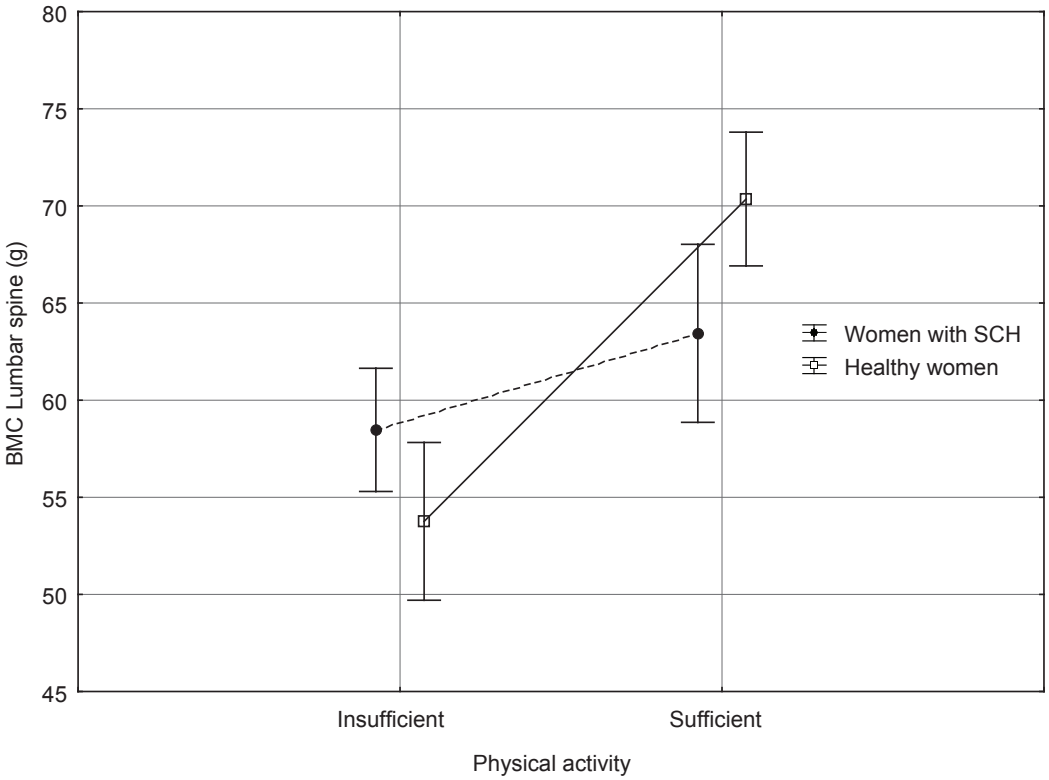


Figure 2. Relationships of BMC lumbar spine, physical activity levels and women's health status (two-way ANOVA results, $F_{1, 131} = 8.8964$, $p = 0.003$), vertical lines – 0.95 CI – confidence intervals

[35]. Nagata et al. studied bone quality using quantitative ultrasound measurement in patients with subclinical hypothyroidism. Their results suggested that hypothyroidism affects bone structure [27]. A hypothesis has emerged that hypothyroidism may impair bone turnover. The most likely cause was a reduction in both osteoclastic bone resorption and osteoblastic activity. However, data on bone mineral status in hypothyroidism are still incomplete and vary between studies. At present, there are no complete data showing any relationship between BMD in adults and hypothyroidism [12]. An earlier Polish study showed that hypothyroidism in premenopausal women does not affect bone density [33].

This study of Polish women with SCH found decreased serum calcium and low bone density. Similar results were presented by Demartini et al. [13] Their survey included 122 patients with congenital hypothyroidism and 153 healthy people in the control group. The study demonstrated that compared to the controls, the patients with subclinical hypothyroidism had a significantly higher incidence of osteopenia and lower levels of serum calcium [13].

This study indicated poorer bone mineralization of the hip and lumbar spine in women with SCH compared to he althy women of the same age. There was also a significantly higher incidence of osteopenia and osteoporosis at the femoral neck in women with thyroid disorders. Women with subclinical hypothyroidism (SCH) had significantly higher levels of bone resorption markers and lower levels of serum calcium and two vitamin D fractions. The present study also analyzed the effect of weekly physical activity based on MET on bone parameters in the women studied. Among both women with SCH and healthy participants, sufficient levels of physical activity according to WHO recommendations were correlated with higher BMD at the hip and BMC at the lumbar spine.

A review of current research shows that an active lifestyle is associated with better BMD and bone status in various parts of the skeleton in postmenopausal women [10, 20, 26]. Physical activity and widely available forms of movement are considered to be a low-cost strategy for the protection of musculoskeletal health and prevention of bone diseases in women [4, 10, 17]. The data indicate that an active lifestyle and habits of daily physical activity can help prevent decreases in postmenopausal bone mineral density [26]. It has been shown in a two-year observational study of menopausal women that physical activity was the most significant factor in improving BMD in both the lumbar spine and the femoral neck [22]. Cross-sectional studies of women with menopausal status confirmed these results. These studies have shown that BMD in the femur and lumbar spine was significantly higher in physically active women compared to the inactive group [19]. There is evidence that exercise, especially resistance and weight training, stimulates bone osteogenesis. The mechanism of this effect consists in stimulating bone mass growth and increasing the thickness and resistance of the cortical bone [5].

However, most studies evaluate the effects of physical activity on the skeletal system in healthy women or those with osteoporosis. There is a lack of studies on the effect of physical activity on BMD in women with SCH. Subclinical hypothyroidism (SCH) may have adverse effects similar to hypothyroidism. The presence of symptoms and reduced physical performance in SCH patients may contribute to an inactive sedentary lifestyle. Tanriverdi et al. [32] conducted analyses of physical activity levels between women with newly diagnosed SCH and healthy controls. In women with SCH, physical activity duration, the number of steps, and muscle strength were significantly lower compared to healthy controls. This study, similar to that conducted by Tanriverdi et al. [32], showed that women with SCH had significantly lower physical activity (by 206.8 MET min./week) than healthy women. Tanriverdi et al [32] points to possible reasons for reduced PA in SCH patients such as the presence of neuromuscular symptoms and exercise intolerance in SCH, frequent weakness, fatigue and reduced functional exercise capacity. There is a lack of data in the literature regarding the level of physical activity in SCH patients, which indicates the need to undertake research in this area.

This study showed that sufficient levels of physical activity had a protective effect on femoral neck BMD and lumbar spine BMC in women. In both groups of women (with SCH and healthy participants), sufficient levels of physical activity significantly determined higher BMD. Physical activity is a recognized important determinant of women's health at all ages [2, 28].

This study included a relatively small number of women with SCH and only of postmenopausal age. The study should be repeated in the future to extend to another group of women with thyroid and parathyroid diseases. It is also worth extending the research in the future to include the analysis of more factors affecting bone health, such as diets, eating habits, and sedentary lifestyles.

Conclusion

In conclusion, women with SCH had statistically significant lower BMD in the femoral neck and lumbar spine and higher values of markers of bone resorption (ICTP, μg/l) than healthy women of the same age. This condition of bone tissue can increase the risk of developing osteoporosis and fractures. Physical activity can be an important area of rehabilitation and physical therapy as an available measure to counteract BMD loss in postmenopausal healthy women and those with SCH. The results of this study indicate the need for preventive diagnosis of bone

mineral status in women with SCH, which can help reduce the risk of osteoporosis at a later age. It is necessary to educate women about the positive impact of a sufficient level of physical activity on bone health. The results of the study confirm the important role of physical activity in maintaining bone health in both groups of women (healthy and those with SCH). Research should continue to verify the reasons for the lower physical activity of women with SCH compared to healthy women. In conclusion, the role of screening bone tissue status and levels of bone turnover markers in people with SCH should be emphasized.

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References

1. Abrahamsen B., Jørgensen H.L., Laulund A.S., Nybo M., Bauer D.C., Brix T.H., Hegedüs L. (2015) The excess risk of major osteoporotic fractures in hypothyroidism is driven by cumulative hyperthyroid as opposed to hypothyroid time: an observational register-based time-resolved cohort analysis. *J. Bone. Miner. Res.,* 30(5): 898–905. DOI: 10.1002/jbmr.2416.
2. Akkurt M., Ökmen M.Ş., Polat M. (2023) Effects of eight-week aerobic exercises combined with resistance training on cardiovascular risk factors in women. *Biomed. Hum. Kinet.,* 15(1): 1–8. DOI: 10.2478/bhk-2023-0001.
3. Bassett J.H.D., Williams G.R. (2016) Role of Thyroid Hormones in Skeletal Development and Bone Maintenance. *Endocrine. Rev.,* 37(2): 135–187. DOI: 10.1210/er.2015-1106.
4. Beck B.R., Daly R.M., Singh M.A., Taaffe D.R. (2017) Exercise and sports science Australia (ESSA) position statement on exercise prescription for the prevention and management of osteoporosis. *J. Sci. Med. Sport.,* 20: 438–445. DOI: 10.1016/j.jsams.2016.10.001.
5. Benedetti M.G., Furlini G., Zati A., Mauro G.L. (2018) The Effectiveness of Physical Exercise on Bone Density in Osteoporotic Patients. *Biomed. Res. Int.,* DOI: 10.1155/2018/4840531.
6. Bertoli A., Fusco A., Andreoli A., Magnani A., Tulli A., Lauro D., De Lorenzo A. (2002) Effect of subclinical hypothyroidism and obesity on whole-body and regional bone mineral content. *Horm. Res.,* 57(3-4): 79–84. DOI: 10.1159/000057956.
7. Bhattoa H.P., Cavalier E., Eastell R., Heijboer A.C., Jørgensen N.R., Makris K., Ulmer C.Z., Kanis J.A., Cooper C., Silverman S.L., Vasikaran S.D. IFCC-IOF Committee for Bone Metabolism (2021) Analytical considerations and plans to standardize or harmonize assays for the reference bone turnover markers PINP and β-CTX in blood. *Clin. Chim. Acta.,* 515: 16–20. DOI: 10.1016/j.cca.2020.12.023.
8. Bolamperti S., Villa I., Rubinacci A. (2022) Bone remodeling: an operational process ensuring survival and bone mechanical competence. *Bone. Res.,* 10: 48. DOI: 10.1038/s41413-022-00219-8.
9. Cosman F., de Beur S.J., LeBoff M.S., Lewiecki E.M., Tanner B., Randall S., Lindsay R. (2014) Clinician's Guide to Prevention and Treatment of Osteoporosis. *Osteoporos. Int.,* 25: 2359–2381. DOI: 10.1007/s00198-014-2794-2.
10. Daly R.M., Dalla Via J., Duckham R.L., Fraser S.F., Helge E.W. (2019) Exercise for the prevention of osteoporosis in postmenopausal women: an evidence-based guide to the optimal prescription. *Braz. J. Phys. Ther.,* 23: 170–180. DOI: 10.1016/j.bjpt.2018.11.011.
11. Delaisse J.M., Andersen T.L., Kristensen H.B., Jensen P.R., Andreasen C.M., Søe K. (2020) Re-thinking the bone remodeling cycle mechanism and the origin of bone loss. *Bone,* 141: 115628. DOI: 10.1016/j.bone.2020.115628.
12. Delitala A.P., Scuteri A., Doria C. (2020) Thyroid Hormone Diseases and Osteoporosis. *J. Clin. Med.,* 9(4): 1034. DOI: 10.3390/jcm9041034.
13. Demartini Ade A., Kulak C.A., Borba V.C., Cat M.N., Dondoni R.S., Sandrini R., Nesi-França S., Lacerda Filho L.D. (2007) Bone mineral density of children and adolescents with congenital hypothyroidism. *Arq. Bras. Endocrinol. Metabol.,* 51(7): 1084–1092. Portuguese. DOI: 10.1590/s0004-27302007000700010.
14. El Hadidy H.M., Ghonaim M., El Gawad S., El Atta M.A. (2011) Impact of severity, duration, and etiology of hyperthyroidism on bone turnover markers and bone mineral density in men. *BMC Endocr. Disord.,* 11: 15. DOI: 10.1186/1472-6823-11-15.
15. Hall J.G., Allanson J.E., Gripp K.W., Slavotinek A.M. (2007) Handbook of Physical Measurements. Oxford University Press.
16. Herbert A.J., Williams A.G., Hennis P.J., Erskine R.M., Sale C., Day S.H., Stebbings G.K. (2019) The interactions of physical activity, exercise and genetics and their associations with bone mineral density: implications for injury risk in elite athletes. *Eur. J. Appl. Physiol.,* 119(1): 29–47. DOI: 10.1007/s00421-018-4007-8.
17. Kemmler W., Bebenek M., Kohl M., Von Stengel S. (2015) Exercise and fractures in postmenopausal women. Final results of the controlled erlangen fitness and osteoporosis prevention study (EFOPS). *Osteoporos. Int.,* 26: 2491–2499. DOI: 10.1007/s00198-015-3165-3.
18. Khundmiri S.J., Murray R.D., Lederer E. (2016) PTH and Vitamin D. *Compr. Physiol.,* 15;6(2): 561–601. DOI: 10.1002/cphy.c140071.
19. Kim K.M., Choi S.H., Lim S., Moon J.H., Kim J.H., Kim S.W., Jang H.C, Shin C.S. (2014) Interactions between dietary calcium intake and bone mineral density or bone geometry in a low calcium intake population

(KNHANES IV 2008–2010). *J. Clin. Endocrinol. Metab.,* 99(7): 2409–2417. DOI: 10.1210/jc.2014-1006.
20. Kopiczko A. (2020) Determinants of bone health in adults Polish women: The influence of physical activity, nutrition, sun exposure and biological factors. *PLoS One,* 15(9): e0238127. DOI: 10.1371/journal.pone.0238127.
21. Kopiczko A., Cieplińska J. (2022) Forearm bone mineral density in adult men after spinal cord injuries: impact of physical activity level, smoking status, body composition, and muscle strength. *BMC Musculoskelet. Disord.,* 23: 81. DOI: 10.1186/s12891-022-05022-4.
22. Kumar A., Mittal S., Orito S., Ishitani K., Ohta H. (2010) Impact of dietary intake, education, and physical activity on bone mineral density among North Indian women. *J. Bone Miner. Metab.,* 28: 192–201. DOI: 10.1007/s00774-009-0118-y.
23. Kuo T.R., Chen C.H. (2017) Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark. Res.,* 5: 18. DOI: 10.1186/s40364-017-0097-4.
24. Lee P.H., Macfarlane D.J., Lam T.H., Stewart S.M. (2011) Validity of the international physical activity questionnaire short form (IPAQ-SF): A systematic review. *Int. J. Behav. Nutr. Phys. Act.,* 8: 115. DOI: 10.1186/1479-5868-8-115.
25. Li X.P., Li X.Y., Yang M.H., Zhu S.W., Wu X.B., Zhang P. (2021) Changes of bone turnover markers after elderly hip fracture surgery. *J. Bone. Miner. Metab.,* 39(2): 237–244. DOI: 10.1007/s00774-020-01140-y.
26. Muir J.M., Ye C., Bhandari M., Adachi J.D., Thabane L. (2013) The effect of regular physical activity on bone mineral density in post-menopausal women aged 75 and over: a retrospective analysis from the Canadian multicentre osteoporosis study. *BMC Musculoskelet. Disord.,* 14: 253. DOI: 10.1186/1471-2474-14-253.
27. Nagata M., Suzuki A., Sekiguchi S., Ono Y., Nishiwaki-Yasuda K., Itoi T., Yamamoto S., et al. (2007) Subclinical hypothyroidism is related to lower heel QUS in postmenopausal women. *Endocr. J.,* 54(4): 625–630. DOI: 10.1507/endocrj.k06-201.
28. Nyrć M., Lopuszanska-Dawid M. (2023) Physical fitness and somatic structure in adolescent taekwondo athletes and untrained peers. *Biomed. Hum. Kinet.,* 15: 63–73. DOI: 10.2478/bhk-2023-0009.
29. O'Connor K.A., Holman D.J., Wood J.W. (2001) Menstrual cycle variability and the perimenopause. *Am. J. Hum. Biol.,* 13: 465–478. DOI: 10.1002/ajhb.1078.
30. Segna D., Bauer D.C., Feller M., Schneider C., Fink H.A., Aubert C.E., Collet T.H., da Costa B.R., et al. (2018) Thyroid Studies Collaboration. Association between subclinical thyroid dysfunction and change in bone mineral density in prospective cohorts. *J. Intern. Med.,* 283(1): 56–72. DOI: 10.1111/joim.12688.
31. Shetty S., Kapoor N., Bondu J.D., Thomas N., Paul T.V. (2016) Bone turnover markers: Emerging tool in the management of osteoporosis. *Indian. J. Endocrinol. Metab.,* 846–852. DOI: 10.4103/2230-8210.192914.
32. Tanriverdi A., Ozcan Kahraman B., Ozsoy I., Bayraktar F., Ozgen Saydam B., Acar S., Ozpelit E., Akdeniz B., Savci S. (2019) Physical activity in women with subclinical hypothyroidism. *J. Endocrinol. Invest.,* 42(7): 779–785. DOI: 10.1007/s40618-018-0981-2.
33. Tuchendler D., Bolanowski M. (2013) Assessment of bone metabolism in premenopausal females with hyperthyroidism and hypothyroidism. *Endokrynol. Pol.,* 64(1): 40–44.
34. Vasikaran S., Eastell R., Bruyère O., Foldes A.J., Garnero P., Griesmacher A., McClung M., Morris H.A., et al. (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards. *Osteoporos. Int.,* 22: 391–420. DOI: 10.1007/s00198-010-1501-1.
35. Vestergaard P., Mosekilde L. (2002) Fractures in patients with hyperthyroidism and hypothyroidism: A nationwide follow-up study in 16,249 patients. *Thyroid.,* 12(5): 411–419. DOI: 10.1089/105072502760043503.
36. Vestergaard P., Mosekilde L. (2003) Hyperthyroidism, bone mineral, and fracture risk–a meta-analysis. *Thyroid.,* 13(6): 585–593. DOI: 10.1089/105072503322238854.
37. Wallace B.A., Cumming R.G. (2000) Systematic review of randomized trials of the effect of exercise on bone mass in pre- and postmenopausal women. *Calcif. Tissue. Int.,* 67(1): 10–18. DOI: 10.1007/s002230001089.
38. Yan Z., Huang H., Li J., Wang J. (2016) Relationship between subclinical thyroid dysfunction and the risk of fracture: A meta-analysis of prospective cohort studies. *Osteoporos. Int.,* 27(1): 115-125. DOI: 10.1007/s00198-015-3221-z.

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