

# Association between vitamin D and bone mineral density in post-menopausal women with metabolic syndrome

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**Background.** The aim of this study was to identify the relation between vitamin D level and mineral bone density in post-menopausal women with metabolic syndrome.

**Materials and methods.** This study included 100 post-menopausal women at age between 50 and 65 with metabolic syndrome. All participants underwent anthropometric measurements. Laboratory tests were performed to determine lipid profile, serum glucose, creatinine, C-reactive protein, vitamin D (25(OH) D), ionized calcium concentration and urine albumin / creatinine ratio. Bone mineral density of the lumbar spine (L1–L4) and total hip was measured by dual-energy X-ray absorptiometry.

**Results.** According to the vitamin D concentration level in the blood all women were divided into four groups: the average failure was observed in 57%, mild failure in 33%, severe failure in 5%; and only 5% of women had normal vitamin levels. The mean 25(OH) D level was  $47.40 \pm 16.91$  nmol/l. According to bone densitometry we found that 77% of all participants had normal bone mineral density, 22% had osteopenia and 5% were diagnosed with osteoporosis. No correlation was found between bone mineral density and 25(OH) D levels. We found a weak positive correlation between high density lipoprotein cholesterol and 25(OH) D ( $r = 0.3$ ,  $p < 0.05$ ) but no significant difference between 25(OH) D and other lipoproteins, calcium ions, glucose, C-reactive protein and urine albumin / creatinine ratio.

**Conclusions.** Hypovitaminosis D is very common among post-menopausal women with metabolic syndrome. No relation was found between the 25(OH) D level and the bone mineral density.

**Key words:** vitamin D, bone mineral density, metabolic syndrome

## INTRODUCTION

It is well known that vitamin D plays an important role in maintaining adequate serum calcium, phosphate concentrations and healthy bone structure. Decrease of bone mineral density (BMD) is an important world-wide health problem, particularly

affecting post-menopausal women (1). Currently there is an increasing interest in the extra-skeletal roles of vitamin D for health and well-being: vitamin D deficiency has been associated with obesity, cardiovascular disease, diabetes mellitus and mental health (2). However, effects of vitamin D supplementation on health are uncertain.

25(OH) D is one of the two vitamin D forms. Vitamin D is actively involved in formation of bone tissue, but it is unknown if diminution of vitamin D changes BMD in post-menopausal women with

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metabolic syndrome. In this study we hypothesize that vitamin D deficit is related with BMD among post-menopausal women. Vitamin D and calcium have long been regarded as a fundamental part of the prevention and treatment of osteoporosis. Findings from observational studies show inconsistent associations between BMD and vitamin D status. The study conducted among Italian elderly women has revealed a positive correlation between BMD and parathyroid hormone, however, no association between BMD and 25(OH) D has been found (3). Recent systematic review and meta-analysis have shown that widespread use of vitamin D for osteoporosis prevention in community-dwelling adults without specific risk factors for vitamin D deficiency seems to be inappropriate (4).

Our study target was patients with metabolic syndrome because association between metabolic syndrome and bone health is uncertain. There are data that women with metabolic syndrome have higher BMD at the hip and spine (5). This finding suggests that metabolic syndrome may have a positive effect for BMD. Recent meta-analysis has shown that metabolic syndrome is a risk factor for appearance of osteoporosis among men, but not women (6). There is a controversy regarding vitamin D deficiency and bone health. The aim of this cross-sectional study is to identify the relation between vitamin D level and BMD in post-menopausal women with metabolic syndrome.

## MATERIALS AND METHODS

**Study design.** This is a cross-sectional study designed to identify the relation between vitamin D level and BMD. The inclusion criteria were the following: age 50–65, diagnosed metabolic syndrome and post-menopausal period. Metabolic syndrome was diagnosed if at least 3 of 5 symptoms written below were present: 1) waist circumference >88 cm; 2) systolic blood pressure (SBP)  $\geq 130$  mmHg and / or diastolic blood pressure (DBP)  $\geq 85$  mmHg; 3) fasting glucose concentration  $\geq 5.6$  mmol/l or a patient has type 2 diabetes mellitus; 4) triglycerides (TG) concentration  $\geq 1.7$  mmol/l; 5) high density lipoprotein (HDL) cholesterol concentration  $< 1.2$  mmol/l in women (7). The women were recruited from those who came to the Vilnius University Hospital Santariškių Clinics according to the Lithuanian High Cardiovascular Risk Primary Prevention Program in

2014. 100 women were included in this study until the end of the year. The exclusion criteria were the following: diagnosed coronary disease, malignant disease, kidney or liver failure, permanent arrhythmias, drug-resistant tuberculosis, acute rheumatic fever or rheumatic disease (acute phase), pulmonary arterial hypertension (greater than grade 2), decompensated heart disease, advanced stage of mental illness. Approval was obtained from the Lithuanian Bioethics Committee, and each participant gave a written informed consent.

**Study variables.** All participants filled in a structured questionnaire about demographic and social characteristics, including age, living place (town or countryside) and addictions (smoking, alcohol consumption). All the patients underwent measurements of height, weight, waist circumference and arterial blood pressure. Waist circumference was measured midway between the top of the hip bone and the bottom of the ribs. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared and classified into normal overweight and obese categories (8). Arterial hypertension was determined when SBP was  $\geq 140$  mmHg and / or DBP was  $\geq 90$  mmHg, or diagnosis of hypertension was documented in a medical record. Arterial hypertension was classified as grade 1 hypertension when SBP was 140–159 mmHg and / or DBP 90–99 mmHg, as grade 2 hypertension when SBP was 160–179 mmHg and / or DBP 100–109 mmHg, as grade 3 hypertension when SBP was  $\geq 180$  mmHg and / or DBP  $\geq 110$  mmHg (9). Laboratory tests were performed in the morning after 12 hours fasting, and the following variables were determined: total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, TG, serum glucose, creatinine, C-reactive protein, 25(OH) D and ionized calcium serum concentration. A microalbumin urine test was also performed and urine albumin / creatinine ratio was calculated.

**25(OH) D concentration.** The normal values of 25(OH) D are considered to be 75–100 nmol/l. 25 nmol/l or less were considered as vitamin D deficiency, while 25–50 nmol/l and 50–75 nmol/l were considered as modest and mild insufficient (10).

**Bone mineral density.** BMD ( $\text{g}/\text{cm}^2$ ) of the lumbar spine (L1–L4) and total hip was measured by dual-energy X-ray absorptiometry (iDXA; GE Lunar, USA). Standardized procedures for participants positioning and scan analysis were used by a certified operator. Osteoporosis was defined as a T-score

equal to or less than ( $-2.5$ ) SD, osteopenia as a T-score that lies between ( $-1$ ) and ( $-2.5$ ) SD (11).

**Statistical analysis.** Statistical analysis was performed using the Microsoft Excel 2010 and SPSS Statistics 17.0. Study variables were described as the mean ( $\pm$ SD) (as median and interquartile range if distribution is abnormal) for quantitative variables or as counts and percentages for qualitative variables. To explain a relationship between 25(OH) D concentration and measured parameters linear regression was applied. The level of significance ( $p$ ) was set to 0.05.

## RESULTS

100 post-menopausal women with metabolic syndrome were included into our study. Demographical characteristics of the population are presented in Table 1. Among all participants only 1% had normal BMI, 26% were overweight and 74% obese, in addition, waist circumference in all participants was higher than 88 cm which refers to abdominal obesity. The mean serum 25(OH) D level was  $47.40 \pm 16.91$  nmol/l (Table 2). A total of 57% had modest insufficiency of 25(OH) D, 33% had mild insufficiency, whereas prevalence of deficiency and sufficiency were both of 5% (Fig. 1). According to bone densitometry and T score we found that 77% of all participants had normal bone mineral density, 22% had osteopenia and 5% were diagnosed with osteoporosis (Fig. 2). In our study we were looking for association between vitamin D and bone mineral density. In this way we divided the total group into the deficiency and modest insufficiency group that was comprised of 62 women and the mild insufficiency and sufficiency group with 38 women. However, there was no significant difference between any of laboratory findings tested including bone mineral density between two 25(OH) D concentration groups (Table 3).

**Table 1.** Demographic characteristics of women with metabolic syndrome

Characteristic*	Data
Age (years), mean ( $\pm$ SD)	$57.57 \pm 3.78$
BMI ( $\text{kg}/\text{m}^2$ ), mean ( $\pm$ SD)	$32.70 \pm 3.9$
Waist circumference (cm), mean ( $\pm$ SD) N = 100	$104.7 \pm 9.55$
Living place: number (%) N = 92	

Town	73 (79)
Countryside	19 (21)
Marital status: number (%) N = 92	
Married currently	62 (67)
Other (divorced, single, widowed)	30 (33)
Education level: number (%) N = 90	
0–12 years	31 (34)
>12 years	59 (66)
Smoking, number (%)	16 (16)
Smoking duration (years), mean ( $\pm$ SD)	$20.4 \pm 7.8$
Arterial hypertension: number (%)	94 (93)
I°	29 (31)
II°	58 (62)
III°	5 (5)

\* If a sample size was not mentioned, N = 100.

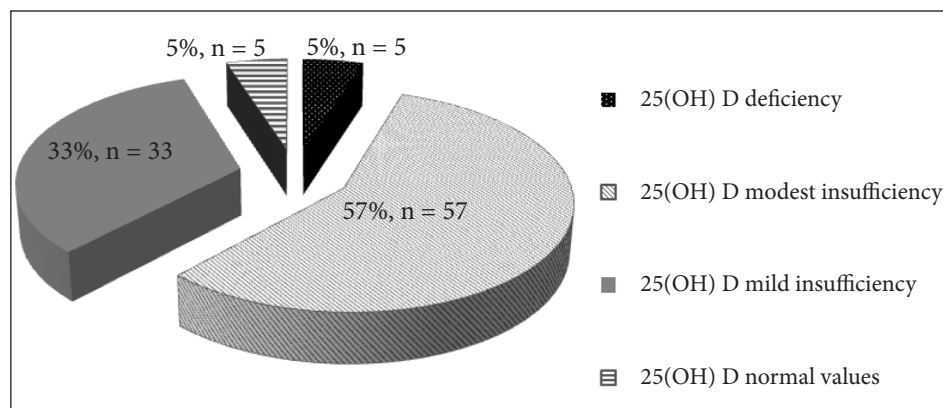
BMI is body mass index.

**Table 2.** Biochemical and bone densitometry parameters of women with metabolic syndrome

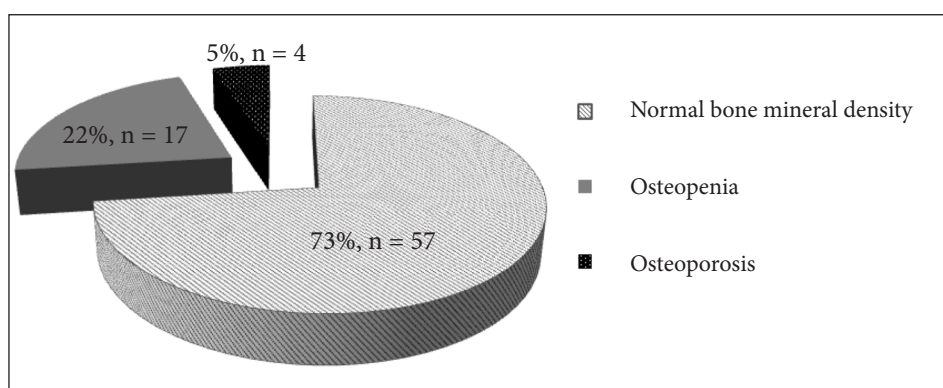
Variable*	Value
<b>Biochemical parameters</b>	
Lipid profile:	
• Total cholesterol (mmol/l), mean ( $\pm$ SD)	6.60 ( $\pm$ 1.56)
• LDL cholesterol (mmol/l), mean ( $\pm$ SD)	4.37 ( $\pm$ 1.36)
• HDL cholesterol (mmol/l), mean ( $\pm$ SD)	1.25 ( $\pm$ 0.24)
• Triglycerides (mmol/l), median (interquartile range)	1.89 (2.56–1.32)
Serum glucose (mmol/l), mean ( $\pm$ SD) N = 99	6.27 ( $\pm$ 1.58)
C-reactive protein (mg/l), median (interquartile range)	2.2 (4.4–1.3)
25-OH D (nmol/l), mean ( $\pm$ SD) N = 100	47.40 ( $\pm$ 16.91)
Ionized Ca (mmol/l), mean ( $\pm$ SD) N = 99	1.18 ( $\pm$ 0.09)
Urine albumin creatinine ratio, median (interquartile range) N = 95	0.57 (1.02–0.43)
<b>Bone densitometry</b>	
BMD of total hip ( $\text{g}/\text{cm}^2$ ), mean ( $\pm$ SD) N = 77	1.08 ( $\pm$ 0.14)
BMD of lumbar spine (L1–L4) ( $\text{g}/\text{cm}^2$ ), mean ( $\pm$ SD) N = 77	1.17 ( $\pm$ 0.18)

\* If a sample size was not mentioned, N = 100.

LDL is low-density lipoprotein, HDL is high-density lipoprotein, BMD is bone mineral density.



**Fig. 1.** Diagram shows the prevalence of 25(OH) D blood status among the study population



**Fig. 2.** Diagram shows the prevalence of osteopenia, osteoporosis and normal bone mineral density among the study population

**Table 3.** Comparisons of biochemical and bone densitometry parameters between two groups with different levels of vitamin D

	Deficiency and modest insufficiency N = 62	Mild insufficiency and sufficiency N = 38	P
<b>Biochemical parameters</b>			
<b>Lipid profile:</b>			
• Total cholesterol (mmol/l), mean (±SD)	6.62 (±1.54)	6.58 (±1.6)	0.9
• LDL cholesterol (mmol/l), mean (±SD)	4.4 (±1.3)	4.33 (±1.46)	0.79
• HDL cholesterol (mmol/l), mean (±SD)	1.21 (±0.22)	1.3 (±0.27)	0.08
• Triglycerides (mmol/l), median (interquartile range)	1.9 (2.55–1.35)	1.85 (2.63–1.28)	0.98
Serum glucose (mmol/l), mean (±SD) N = 99	6.23 (±1.51)	6.34 (±1.71)	0.75
C-reactive protein (mg/l), median (interquartile range)	2.7 (4.7–1.33)	1.9 (2.8–0.75)	0.04
25-OH D (nmol/l), mean (±SD) N = 100	37.91 (±8.77)	62.87 (±15.55)	
Ionized Ca (mmol/l), mean (±SD) N = 99	1.17 (±0.09)	1.19 (±0.07)	0.4
Urine albumin creatinine ratio, median (interquartile range) N = 95	0.66 (1.05–0.42)	0.54 (1.01–0.44)	0.22
<b>Bone densitometry</b>			
BMD of total hip (g/cm <sup>2</sup> ), mean (±SD) N = 77	1.09 (±0.15)	1.06 (±0.12)	0.37
BMD of lumbar spine (L1–L4) (g/cm <sup>2</sup> ), mean (±SD) N = 77	1.17 (±0.2)	1.17 (±0.15)	0.95

LDL is low-density lipoprotein, HDL is high-density lipoprotein, BMD is bone mineral density.

We checked linear correlations between the 25(OH) D blood concentration and the following groups: smokers and non-smokers, those who lived in the countryside and those who lived in the city, married and non-married. We also compared 25(OH) D values according to education levels, and the month when 25(OH) D concentration has been measured in the blood, but no significant relationships were found. The only one measurement higher in the deficiency group was the mean blood pressure – 107.61 ( $\pm 12.77$ ) if compared to the vitamin D sufficiency group – 102.64 ( $\pm 11.16$ ). In addition, the greater degree of arterial hypertension the participants had, the lower concentration of 25(OH) D was measured in the blood. However, this finding was not significant perhaps because of a small number of cases with grade 3 hypertension. The graphic presentation of these findings is omitted since we chose to present few demographic and laboratory correlations with 25(OH) D (Fig. 3). We found the only one and weak positive correlation between HDL cholesterol and 25(OH) D ( $r = 0.3$ ,  $p < 0.05$ ). This finding leads to the perception that with increase of vitamin D, HDL cholesterol also tends to increase. No significant relation between 25(OH) D and other lipoproteins, calcium ions, glucose, C-reactive protein and urine albumin / creatinine ratio was found.

## DISCUSSION

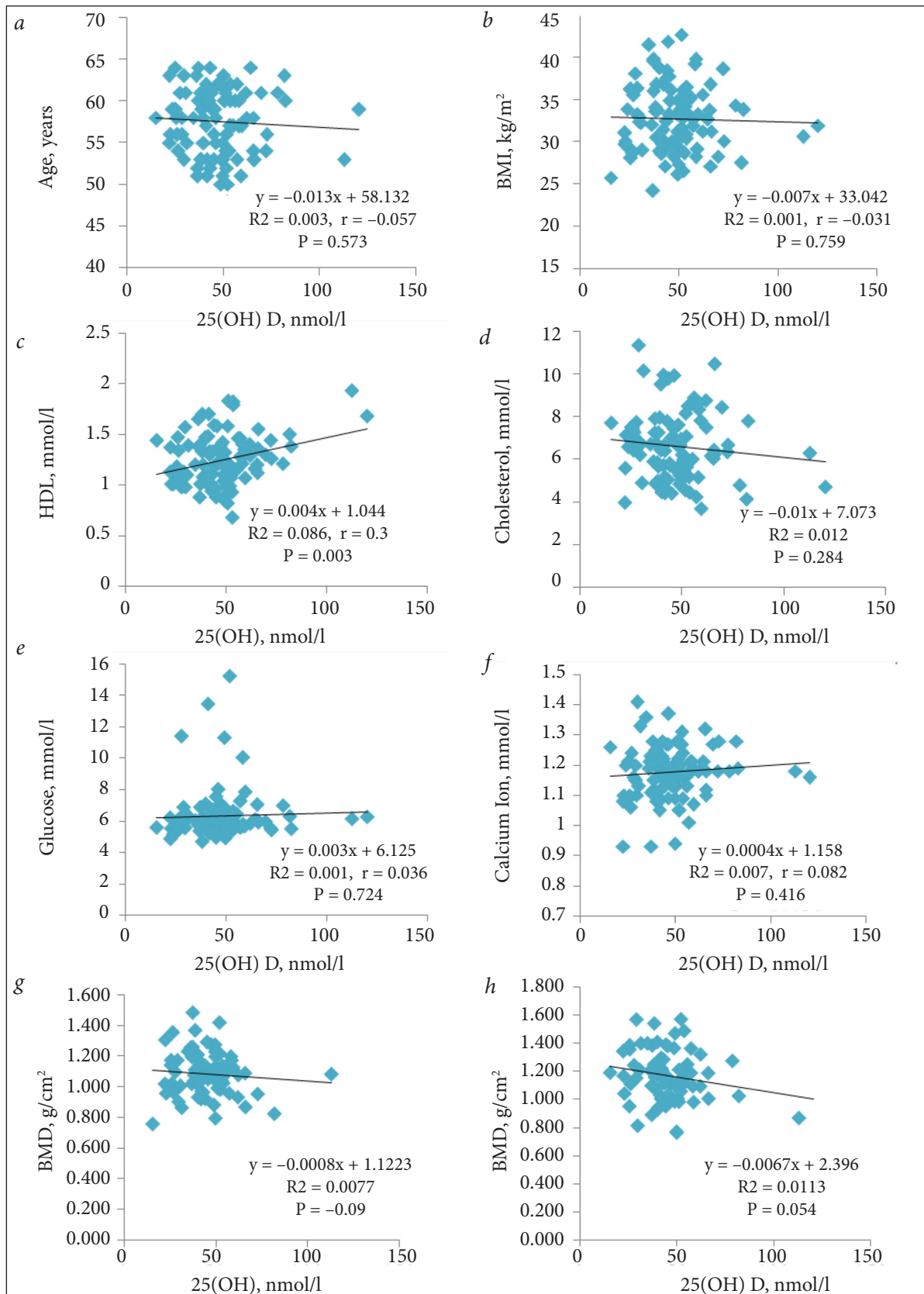
This cross-sectional study has shown that only 5% women had sufficiency of vitamin D while 62% overweight post-menopausal women had deficiency and modest insufficiency of vitamin D and 33% women had mild insufficiency. Vitamin D deficiency and insufficiency is not only found in post-menopausal women in Lithuania, but this problem is also highly prevalent in Lithuanian female school graduates. Only 3.4% young adult females were vitamin D sufficient. Meanwhile, vitamin D deficiency is recognized as a worldwide health problem (12). The leading cause of this problem is lack of the sun exposure. Sunshine is the major source of vitamin D for most humans. Also vitamin D status depends on vitamin D intake through the diet. Synthesis of vitamin D in the human body varies during the year. During winter people living in the northern countries rely on dietary vitamin D and body supplies because sunlight is not strong

enough for vitamin D synthesis in the skin. Although vitamin D deficiency is a common underdiagnosed condition, but there is no direct evidence on the effect of screening for vitamin D on health outcomes (13).

The findings of our study indicated no significant seasonal variations of vitamin D levels. Data for this study have been collected within the period of February–November 2014. Vitamin D concentrations for every participant have been measured only once, thus we could not assess the individual variations of vitamin D levels according to the seasonal changes. The prospective study conducted by Blezgy A. et al in Lithuania revealed changes of vitamin D concentration during the year in the Lithuanian men population (14). The result showed that vitamin D concentration tended to increase for all the participants in the warm season of the year. Our results, however, showed no relationship with timing. Nevertheless, we need to consider the second measurement of vitamin D blood concentration in our population in different time of the year to test for seasonal variations.

According to bone densitometry and its validated measure, T score, we found that 77% of all participants had normal BMD, 22% had osteopenia and 5% were diagnosed with osteoporosis. The prevalence of osteoporosis in the largest countries in the EU is higher. According to Kanis J. A. et al., approximately 21% of women aged 50–84 years are classified as having osteoporosis. There may be small differences in the age- and sex-specific BMD in different European countries as well as within countries (15). The prevalence of osteopenia was, as expected, higher than that of osteoporosis. Ström O. et al. also reported that the prevalence of osteopenia was higher than that of osteoporosis at all ages but does not increase markedly with age. Thus the ratio of individuals with osteopenia to those with osteoporosis varies with age (1).

In our study we were looking for association between vitamin D and BMD. However, there was no significant difference between BMD in the lower and higher 25(OH) D concentration groups. The results are not surprising. Although the relationship between serum 25(OH) D levels and bone health has been explored from a variety of different perspectives, findings from observational studies show inconsistent associations between BMD and vitamin D status (16). Vitamin D supplement effect



**Fig. 3.** Scatter plots showing a relationship between the 25(OH) D concentration in blood and the following factors: *a* – age of participants, *b* – body mass index, *c* – high density lipoprotein concentration in blood, *d* – total cholesterol concentration in blood, *e* – serum glucose concentration, *f* – calcium ion concentration in blood, *g* – bone mineral density of femoral neck, *h* – bone mineral density of lumbar spine

on BMD is also controversial. Jackson R. D. et al. reported results from the Women's Health Initiative (WHI) calcium plus vitamin D trial. 36,000 post-menopausal women were included. The results have shown that calcium with vitamin D supplementation increased total-hip BMD by 1% as compared with placebo (17). Recent meta-analysis has shown no significant impact of vitamin D supplement on BMD. The negative findings have shown that perception that vitamin D works directly on bone cells to promote mineralization is probably incorrect (4).

Some studies performed in Lithuania were designed to assess a correlation between 25(OH) D and BMD. Strazdienė V. et al. have not found any association between vitamin D and BMD of the lumbar spine in elderly women, only a weak positive correlation with BMD of all body, the neck of the femur and the upper part of the femur (18). The previous study of Strazdienė V. et al. also showed only a weak positive correlation between 25(OH) D and BMD of all regions except the lumbar spine (19).

In this small group of participants we found an unexpected relation between vitamin D and high density lipoprotein cholesterol, not directly related to the object of this study. This finding leads to the perception that with increase of vitamin D, HDL cholesterol also trends to increase. If it is so, it may lead to some preventive perspectives in future when administering vitamin D for selected population.

The limitations of our study were that the study population included only women. Therefore the results cannot be generalized to the entire population. Another limitation was a relatively small sample size, and for this reason some potential significant relationship between vitamin D blood concentration and other parameters may have not been found. Finally, the second measure of vitamin D for the same person after half a year period may spread more light on our findings.

## CONCLUSIONS

Our study showed only 5% of women having sufficient level of vitamin D while the rest had mild, modest or deep insufficiency of vitamin D. No relation was found between 25(OH) D level and bone mineral density in post-menopausal women with metabolic syndrome. We found a weak pos-

itive correlation between high density lipoprotein cholesterol and 25(OH) D. This relationship is important for proper understanding and preventing cardiovascular disease.

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## VITAMINO D IR KAULŲ MINERALŲ TANKIO RYŠYS MOTERIMS PO MENOPAUZĖS, SERGANČIOMS METABOLINIŲ SINDROMŲ

### *Santrauka*

**Darbo tikslas.** Nustatyti ryšį tarp vitamino D koncentracijos kraujyje ir kaulų mineralų tankio moterims po menopauzės, sergančioms metaboliniu sindromu.

**Tyrimo medžiaga ir metodai.** Tyrime dalyvavo šimtas 50–65 metų amžiaus moterų po menopauzės, kurioms diagnozuotas metabolinis sindromas. Visoms tiriamosioms atlikti antropometriniai matavimai ir šie laboratoriniai tyrimai: lipidograma, gliukozės, kreatinino, C-reaktyviojo baltymo, vitamino D (25(OH) D) koncentracija kraujo serume, jonizuotas kalcis ir albumino / kreatinino santykis šlapime. Įvertintas kaulų mineralų tankis juosmeninėje stuburo dalyje (L1–L4) ir šlaunikaulyje naudojant dvigubos energijos rentgeno spindulių absorbcimetriją.

**Tyrimo rezultatai.** Pagal vitamino D koncentraciją kraujyje tiriamosios suskirstytos į keturias grupes: vidutinis nepakankamumas nustatytas 57 % moterų, lengvo laipsnio nepakankamumas – 33 %, sunkus – 5 %, o normali vitamino koncentracija buvo tik 5 % moterų. 25(OH) D vidurkis –  $47,40 \pm 16,91$  nmol/l. Įvertinę densitometrijos rezultatus, nustatėme, kad 77 % tiriamųjų kaulų mineralų tankis buvo normalus, 22 % diagnozuota osteopenija, o 5 % – osteoporozė. Tyrime buvo nustatytas patikimas ryšys tarp vitamino D ir didelio tankio lipoproteinų cholesterolio koncentracijų kraujyje ( $r = 0,3$ ,  $p < 0,05$ ). Nenustatėme koreliacijos tarp vitamino D koncentracijos ir kaulų masės tankio, kitų lipoproteinų, kalcio jonų, gliukozės, C-reaktyvaus baltymo kraujyje, albumino / kreatinino santykio šlapime.

**Išvados.** Moterims po menopauzės, kurioms nustatytas metabolinis sindromas, vitamino D hipovitaminozė yra labai paplitusi ir tik 5 % moterų nustatyta normali vitamino D koncentracija. Šioje tiriamųjų grupėje nenustatytas patikimas vitamino D hipovitaminozės ir kaulų mineralų tankio ryšys.

**Raktažodžiai:** vitaminas D, kaulų mineralų tankis, metabolinis sindromas