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ES ENDOCRINOLOGY AND METABOLIC DISEASES

“COMPARATIVE CHARACTERISTICS OF METABOLIC MARKERS IN ASSESSMENT OF POSTMENOPAUSAL BONE HEALTH”

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ABSTRACT

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Contents

Abbreviations

ACEi - angiotensin-converting enzyme inhibitors
AH - arterial hypertension
AP - alkaline phosphatase
ARB - angiotensin receptor blocker
BB - beta blocker
BG - blood glucose
BMD - bone mineral density
BMI - body mass index
BP - blood pressure
BW - body weight
CRP - C-reactive protein
CVD - cardiovascular disease
DBP - diastolic blood pressure
DEXA - double-energy X-ray absorptiometry
DPD / Cr - Pyrilnks D / creatinine in urine
eGFR - glomerular filtration
FRAX score - a calculator for assessing the risk of fracture
HDL - high density lopoproteins
HF - hip fracture
HOMA-IR - the homeostasis model for the assessment of insulin resistance
IR - insulin resistance
LDL - low density lopoproteins
MBRs - markers for bone remodeling
MetS - metabolic syndrome
MOF - major osteoporotic fractures
MP - menopause
MSC - mesenchymal stem cells
nAH - newly diagnosed AH
OC - osteocalcin
OGTT - oral glucose tolerance test
PTH - parathyroid hormone SBP - systolic BP
TBS - trabecular bone score
TC - total cholesterol
TG - triglycerides
TNF alpha - tumor necrosis factor alpha
T2DM - type 2 diabetes mellitus
WC - waist circumference
25(OH)D - 25-hydroxy vitamin D

1. Introduction

Osteoporosis is the most common metabolic bone disease worldwide. It is an escalating public health problem. However, it is often not diagnosed and treated in a timely manner, as it is clinically asymptomatic before fractures occur. This skeletal disorder has a multifactorial genesis that develops on the basis of genetic and environmental factors. It is due to an imbalance between osteoblastic bone formation and osteoclastic bone resorption. Conditions for the development of osteoporosis are present in all individuals after middle age, and this leads to an increased risk of fractures. Fractures, in turn, are associated with high morbidity, mortality and significant health care costs. In this regard osteoporosis and related fractures represent a significant burden for both the individual and society. It is clear that women are more affected by this health problem. One of the reasons is that women have lower bone mass than men. On the other hand, there is a process of rapid bone loss in women after the onset of menopause (MP).

Metabolic syndrome (MetS) is another very common and socially significant global problem, also associated with increased morbidity and mortality, as it is a composite of risk factors that predispose to the development of type 2 diabetes mellitus (T2DD) and cardiovascular diseases. It is undoubted that the risk of the development of MetS, as in osteoporosis, increases with age. In this regard, the economic burden of both health problems will increase in the future due to the increase in life expectancy and the proportion of elderly subjects. It is clear that the components of the syndrome adversely affect the cardio-metabolic profile. However, little is known about the factors that determine the possible relationships between bone health and MetS.

The association between osteoporosis and MetS have been long discussed, but the results of numerous clinical studies remain controversial. It is clear that the relationships between metabolic changes and bone integrity are complex and diverse. It is likely that the different number, combination and degree of manifestation of the MetS components in each individual may contribute to these inconsistent outcomes. However, bones are not inert structures. They are relatively dynamic tissue that could affect metabolic processes in the body. In this regard, the relationships between bone integrity and MetS should be considered in two directions.

Due to the conflicting results of the clinical trials on the relationship between two socially significant health problems, we decided to conduct a cross-sectional study to analyze possible associations of MetS and its components with bone health in postmenopausal women.

2. Purpose and tasks

2.1 Objective: To establish a possible association between bone health in postmenopausal women and their metabolic profile by comparing certain metabolic parameters and inflammatory markers and to assess the relationship between metabolic syndrome and bone integrity.

2.2 Tasks: 1. Evaluation of bone health in postmenopausal women by BMD measurement at the level of lumbar spine and proximal femur, assessment of bone metabolic markers and calculation of fracture risk. 2. Evaluation and analysis of the relationship between age and duration of MP with bone health. 3. Evaluation and analysis of the relationship between some anthropometric, hormonal, metabolic and inflammatory parameters with bone health in postmenopausal age. 4. Evaluation and analysis of the relationship between blood pressure and the intake of antihypertensive drugs with bone health in postmenopausal age. 5. Evaluation and analysis of the complex relationship between metabolic syndrome and bone health in postmenopausal age. 6. Determination of risk profile for compromised bone health in postmenopausal age.

3. Materials and methods

3.1 Selection of participants: In the period 09.2019 - 10.2020, postmenopausal women aged > 45 years from Northeastern Bulgaria were selected for the study. The subjects were selected during their hospitalization in the Clinic of Endocrinology and Metabolic Diseases of the University Hospital "St. Marina" Varna or after an examination in the consulting room of the clinic.

Inclusion criteria: 1. Women with pre-signed informed consent to be included in the study; 2. Postmenopausal women with a missing menstrual cycle of ≥ 1 years. Exclusion criteria: 1. Women without signed informed consent to be included in the study; 2. Iatrogenic and premature MP (< 40 years); 3. Current or past use of drugs that affect bone metabolism (hormone therapy,

corticosteroids, anticonvulsants, drugs for treatment of osteoporosis); 4. History of malignancy; 5. Acute and chronic inflammation; 6. Thyroid and parathyroid dysfunction; 7. Limited physical activity (immobilization, paralysis); 8. Liver and / or kidney disease; 9. Known diabetes mellitus and / or taking antidiabetic medications.

3.2 Clinical methods: Participants underwent a structured medical interview and medical examination. Height (cm), body weight (BW) (kg) and waist circumference (WC) (cm) were determined. Blood pressure (BP) was measured with a calibrated sphygmomanometer on the dominant arm when the subjects were in sitting position, 10 minutes after rest. The mean values of three measurements performed at interval of 5 minutes were used for analysis. Depending on the presence and the degree of the target organs damages according to the WHO classification, the participants with arterial hypertension (AH) were divided into 3 groups: Stage I AH; Stage II AH; Stage III AH.

3.3 Laboratory methods: Blood samples for analysis of hormonal, metabolic and inflammatory parameters, as well as bone metabolic markers were taken in the morning in fasting state after at least 8 hours without intake of foods and fluids containing calories. Part of the venous blood samples were taken in ice, then centrifuged at 4000 rpm for 15 minutes, and the separated serum was stored at -70 ° C until the analysis of the bone formation marker osteocalcin (OC) and the proinflammatory cytokine TNF alfa. The level of Ca^{2+} was determined from capillary blood taken in the morning in fasting state. A sample of the first portion of morning urine was also taken and stored at -70 ° C until the analysis of urinary marker of bone resorption pyrilinks D in combination with urinary creatinine level. The laboratory analysis of the studied parameters was conducted in the Central Clinical Laboratory of the University Hospital "St. Marina", Varna.

Analyzed laboratory parameters: Biochemical parameters: Blood glucose: determined enzymatically by the hexokinase method (Olympus and Advia 1800). Lipid profile: determined by enzymatic calorimetry method with direct determination of HDL-C and calculation of LDL-C (Olympus and Advia 1800). Uric acid level: determined by uricase enzyme method (Olympus and Advia 1800 analyzer). Alkaline phosphatase: determined by kinetic

colorimetric method (Olympus and Advia 1800 analyzer). AST and ALT: determined by a modified IFCC two-stage kinetic test of Warburg (Olympus and Advia 1800 analyzer). Creatinine: determined by Jaffe - kinetic method (Olympus and Advia 1800 analyzer) with subsequent calculation of glomerular filtration rate (eGFR) according to the Cockcroft-Gault Equation formula. Serum and urinary calcium: determined by colorimetric method with Aresnazo III (Olympus analyzer). Serum and urinary phosphorus: determined by colorimetric method with molybdenum blue formation (Olympus analyzer). Inflammatory parameters: CRP: determined by immunoturbidimetric analysis (Olympus and Advia 1800 analyzer). Tumor necrosis factor alpha (TNF alfa): Serum levels were measured by ELISA with a ready-made test kit (Diaclone, France). The method takes into account the qualitative and quantitative level of TNF alfa. The tests are performed according to the protocol requirements of the manufacturer. Serum TNF alfa levels were reported in pg/ml (reference ranges 0-8 pg/ml). Bone metabolic markers: Bone formation marker osteocalcin: determined by chemiluminescent immunoassay with Immulite 2000 analyzer. The method determines the amount of intact OC molecules and does not take into account fragmentary forms. The tests were performed according to the protocol requirements of the manufacturer. Serum levels of OC were reported in ng/ml (reference ranges <2.2-22 ng/ml). Bone resorption marker Beta Crosslaps: determined by electrochemiluminescent immunoassay with Cabas 6000 analyzer. This assay quantified all fragments of type I collagen degradation. Serum levels of Beta-crosslaps were reported in ng/ml (reference ranges 0.104-1.008 ng/ml for postmenopausal age). Bone resorption marker pyrilinks D: determined by enzymatic chemiluminescent immunoassay with Immulite 2000 analyzer. The method determines the amount of deoxypyridinoline in urine. The levels of bone resorption marker were reported in nmol/l. In order to correct the variations in the urine flow, it was necessary to determine the concentration of creatinine in the urine, which was reported in mmol/l. In this regard, the results were presented as the ratio of pyrilinks D and urinary creatinine (DPD/Cr). They were reported in nmol/mmol (reference ranges 3.0-7.4 nmol/mmol for postmenopausal women). The level of 25-hydroxy vitamin D (25 (OH) D), TSH, PTH, insulin: determined by chemiluminescent immunoassay of the Immulite 2000 analyzer.

3.4 Functional test: oral glucose tolerance test (OGTT): the test was performed in the morning after at least 8 hours of night fasting, with 75 g of powdered glucose dissolved in 250-300 ml of water, taken for about 5 minutes, in a sitting position. Blood samples were taken at baseline and after 120 min. The HOMA-index (homeostasis model assessment) was calculated according to the formula: fasting serum insulin (mIU/l) × fasting plasma glucose (mmol/l)/22.5.

3.5 Instrumental examinations: The presence or absence of vertebral fractures was assessed by radiography of the lumbar spine in two projections. Measurement of bone mineral density (BMD and T score) at the level of lumbar spine and proximal femur by dual-energy X-ray absorptiometry (DEXA) was performed with a Lunar Prodigy Bx-1L (GE Medical, Medison, WI, USA). The Fracture Risk Assessment Tool (FRAX) (https://www.sheffield.ac.uk) determined the 10-year probability of a hip fracture (HF) and the 10-year probability of a major osteoporotic fracture (MOF). The fracture risk calculation was performed according to formula for Caucasian race using BMD (g/cm^2) of the femoral neck.

3.6 Statistical analysis: Data were processed with SPSS v. 20.0 for Windows. For all performed analyses an acceptable level of significance was $p < 0.05$ with a confidence interval of 95%. Statistical methods: Analysis of variance (ANOVA); Variation analysis; Correlation analysis (Rearson's R; Spearman correlation); Regression analysis - univariate and multifactorial linear step analysis; Risk assessment analysis (OR); ROC curve analysis to determine threshold values; Comparative analysis (evaluation of hypotheses) - χ^2, t-test; Graphic and tabular method of displaying the obtained results.

4. Results and discussion

4.1 Evaluation of bone health: The study included 84 individuals. They were from 46 to 75 years old (mean age in total group 60.54 ± 7.07 years). The time from the onset of MP was between 1 and 24 years (mean duration of MP in total group 11.45 ± 6.62 years).

Evaluation of bone health according to osteodensitometry

According to the results of DEXA, the subjects were divided into three groups by the lowest BMD at the level of the lumbar spine, the mean value from total

neck and/or femoral neck: healthy controls -T-score ± 1 SD, osteopenia - T-score from - 1 to -2.5 SD and osteoporosis - T-score ≤ -2.5 SD (Fig. 1).

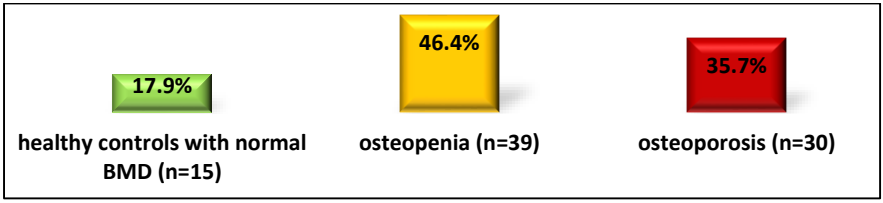

Figure 1. Distribution according to the BMD

The comparative analysis of the assessed clinical and laboratory parameters between the analyzed according to the BMD groups is presented in Table 1. No significant difference was found in terms of smoking and alcohol consumption.

Table 1. Comparative characteristics of the assessed clinical and laboratory parameters between the groups analyzed according to BMD (mean ± SD)

Parameter	**Healthy controls**	**Osteopenia**	**Osteoporosis**	**P value**
Age (y)	56.60±6.58	60.21±6.19	62.93±7.59	**0.015**
Menopause duration (y)	7.56±4.79	9.92±5.72	15.03±6.99	**<0.001**
WC (cm)	94.33±9.34	91.03±12.26	89.33±11.15	0.387
BW (kg)	74.47±7.65	69.92±11.18	65.43±12.08	**0.033**
BMI (kg/m^2)	27.64±3.27	27.13±4.01	26.49±4.56	0.651
Systolic BP (mmHg)	119.33±10.33	124.74±13.18	125.33±9.46	0.225
Diastolic BP (mmHg)	74.33±7.76	76.97±8.61	79.67±6.29	0.076
TSH (μIU/ml)	1.56±1.08	1.75±1.14	1.82±1.07	0.761
PTH (pg/ml)	54.41±20.56	49.49±18.74	51.04±17.37	0.685
25(OH)D (ng/ml)	21.79±9.72	23.51±9.18	21.17±8.95	0.561
Ca (mmol/l)	2.39±0.09	2.43±0.09	2.44±0.09	0.457
Ca^{2+} (mmol/l)	1.20±0.06	1.18±0.07	1.18±0.08	0.707
Ca in urine (mmol/24 ч.)	4.35±3.03	2.95±2.84	3.44±2.36	0.241
P (mmol/l)	1.22±0.16	1.17±0.14	1.15±0.15	0.327
P in urine (mmol/24 ч.)	29.74±21.09	18.47±15.47	17.96±8.93	**0.028**
eGFR (ml/min)	101.67±24.14	91.74±22.31	82.80±23.47	**0.035**
BG – 0 min (mmol/l)	5.64±0.51	5.57±0.54	5.67±0.65	0.773
BG - 120 min (mmol/l)	5.36±1.08	6.06±1.99	6.32±1.78	0.244
Insulin – 0 min (μIU/ml)	10.39±5.11	7.63±3.52	9.12±6.60	0.174

Insulin – 120 min (μIU/ml)	51.85±19.78	46.87±22.27	61.30±52.07	0.253
HOMA index	2.66±1.48	1.91±0.92	2.33±1.79	0.167
TC (mmol/l)	5.43±0.89	5.77±1.37	5.77±0.75	0.568
TG (mmol/l)	1.28±0.62	1.19±0.49	1.09±0.45	0.469
LDL – C (mmol/l)	3.34±0.88	3.53±1.21	3.55±0.75	0.799
HDL – C (mmol/l)	1.57±0.33	1.69±0.44	1.72±0.36	0.485
Uric acid (mcmol/l)	313.5±79.4	275.67±73.7	279.3±66	0.212
TNF-alfa (pg/ml)	2.58±6.81	4.25±6.28	7.70±10.37	**0.015**
CRP (mg/l)	4.16±4.68	3.35±4.66	4.57±8.01	0.703
Creatinine (mcmol/l)	66.27±14.24	64.82±9.06	65.43±11.6	0.908
ASAT (U/l))	22.98±9.82	23.26±12.52	22.51±7.43	0.958
ALAT (U/l)	22.56±10.17	23.43±25.18	19.92±11.59	0.745

Evaluation of bone health according to bone metabolic markers

No significant differences were found from the comparative analysis of the bone metabolic markers between the analyzed according to BMD groups. However, the results of the performed correlation analyses revealed that BMD at the L1-L4 level was negatively associated with OC (for BMD L1-L4 r = -0.223, p = 0.041). The negative relationship between BMD and OC level was maintained after considering age (for BMD L1-L4 r = -0.259, p = 0.018) and duration of MP (for BMD L1-L4 r = -0.261, p = 0.017). On the other hand, the negative relationship between BMD and OC was lost after considering BW, BMI and WC. The results of the correlation analyses with the serum marker of bone resorption revealed that BMD at the level of proximal femur was negatively associated with Beta Crosslaps (for BMD Total Neck r = -0.281, p = 0.01). This dependence was maintained after considering age (for BMD Total Neck r = -0.305, p = 0.05), duration of MP (for BMD Total Neck r = -0.308, p = 0.05), BW (for BMD Total Neck r = - 0.255, p = 0.02) and BMI (for BMD Total Neck r = -0.253, p = 0.021). After WC adjustment, the negative correlation was lost. On the other hand, the results of the correlation and regression analyses between BMD in the studied skeletal areas and DPD/Cr did not demostrate a significant dependence. However, a positive correlation was found between DPD/Cr and eGFR (r = 0.377; p <0.01). Subsequent partial correlation analysis between BMD and DPD/Cr revealed a significant negative relationship only with BMD at the L2 level (for BMD L2 r = -0.223, p = 0.042). Additionally, OC was found to correlate positively with Beta Crosslaps (r =

0.504, p <0.001). On the other hand, no dependences between DPD/Cr with OC and Beta Crosslaps were found.

The higher levels of bone metabolic markers at lower BMD suggest that postmenopausal osteoporosis is associated with higher bone turnover. To distinguish the clinical group, and especially patients with osteoporosis, from healthy controls, we determined cut-off thresholds for OC (5.18 ng/ml (AUC = 0.621 (0.492-0.749), p = 0.006), Beta Crosslaps (0.44 ng/ml (AUC = 0.510 (0.343-0.678), p = 0.008) and the DPD/Cr ratio (5.67 nmol/mmol (AUC = 0.510 (0.355-0.666), p = 0.007) (Fig. 2 a, b, c).

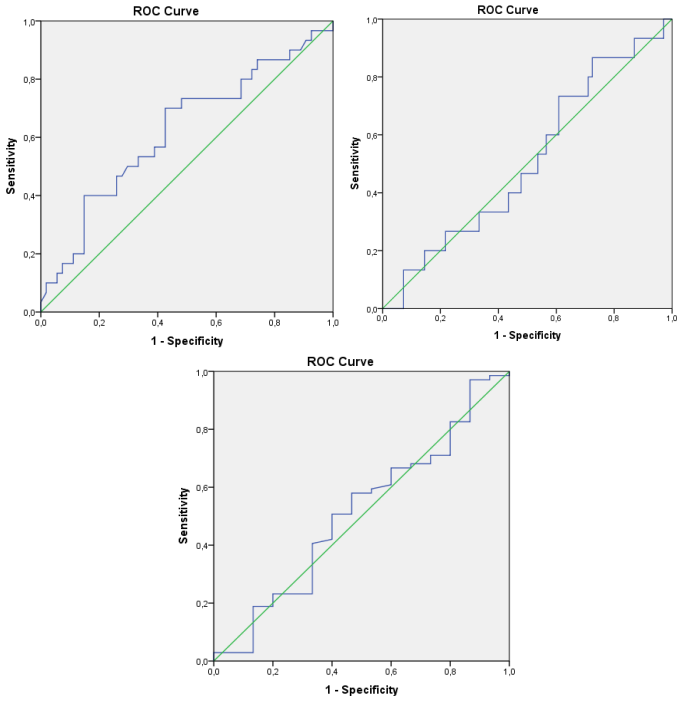

Figure 2 a, b, c. ROC Curve analyses to determine the threshold of osteocalcin, Beta Crosslaps and DPD/Cr ratio

The results above the determined threshold are associated with a decrease in bone density, while the levels of bone markers below these values are considered as optimal.

The threshold value of OC gives 70% accuracy in distinguishing patients with postmenopausal osteoporosis from healthy controls. The DPD/Cr threshold value gives 60% accuracy in distinguishing patients with postmenopausal osteoporosis from healthy controls, while the Beta Crosslaps threshold could be used to differentiate healthy controls from cases with compromised bone density (osteopenia and osteoporosis) and gives 50% accuracy. On the other hand, the combination of threshold values of OC and DPD/Cr give between 75% and 80% probability for postmenopausal osteoporosis, while the combination of threshold values of OC and Beta Crosslaps give only 40% probability.

Fracture risk assessment in the studied groups

From the comparative analysis of the 10-year risk of MOF and HF, as expected, a significant difference was found between the studied groups (Table 2). The results of the analysis confirm that patients with osteoporosis have the highest fracture risk.

Table 2. Comparative analysis of fracture risk in the groups analyzed according to BMD (mean ± SD)

Parameter	Healthy controls	Osteopenia	Osteoporosis	P value
10 year risk of MOF (%)	5.48±1.63	9.02±3.50	12.52±5.42	<0.001
10 year risk of HF (%)	0.30±0.20	1.26±0.99	2.95±2.60	<0.001

The analysis of the 10-year risk of MOF revealed that cut-off thresholds of OC and Beta Crosslaps above the determined values were associated with increased risk (for MOF according to OC 10.37 ± 5.38% to 8.71 ± 3.68%, p = 0.007; for MOF according to Beta Crosslaps 10.15 ± 5.53% to 9.12 ± 3.83%, p = 0.043). Similar results were found in the analysis of the 10-year risk of HF (for HF according to OC 2.09 ± 2.38% to 1.19 ± 1.06%, p = 0.002; for HF according to Beta Crosslaps 1.87 ± 2.13% to 1.52 ± 1.76%, p = 0.264). On the

other hand, the analysis of fracture risks according to the DPD/Cr threshold value did not reveal significant differences.

Evaluation and analysis of Ca-P metabolism in the studied groups

Calcium metabolism: No correlation was found between total serum Ca and urinary calcium with bone density, but a positive relationship was found between Ca^{2+} and BMD ($r = 0.349$; $p < 0.001$) in the studied skeletal areas (Fig. 3). These results suggest that higher, but within the reference range, biologically active calcium in the circulation is associated with higher BMD.

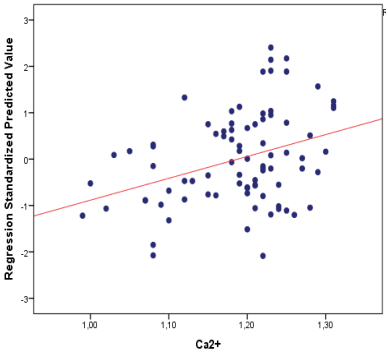

Figure 3. Dependence of BMD (g/cm^2) with Ca^{2+} (mmol/l)

Because of the thiazide intake in part of the observed subjects (32.1%) and the known effects of this class of drugs on calcium metabolism, we compared the levels of Ca in blood and urine between those taking and those not taking thiazide. The results revealed that there was no significant difference in the levels of total serum Ca, Ca^{2+} and calcium in urine between the two groups, but confirmed that the levels of Ca in the urine of patients taking thiazide were lower (3.55 ± 2.55mmol/24h to 3.00 ± 3.09mmol/24h, $p > 0.05$). In addition, analyses revealed that calcium in urine correlated significantly with BMD in subjects taking thiazide, with a strong positive relationship between calcium retention and BMD in the studied skeletal areas ($r = 0.381$; $p = 0.05$). This result suggests that urinary Ca retention has a beneficial effect on bone mass in patients taking thiazide.

A comparison of bone metabolic markers with serum and urinary Ca levels revealed some differences. Initial correlation analyses inicated that total serum Ca was positively associated with OC (r = 0.272; p = 0.012) (Fig. 4 a) and Beta Crosslaps (r = 0.219; p = 0.045) (Fig. 4 b), but the association with DPD/Cr was negative (r = -0.227; p = 0.037) (Fig. 5 a). No significant correlations with bone metabolic markers were found for Ca^{2+}. On the other hand, a significant positive correlation was found between urinary calcium and DPD/Cr (r = 0.265; p = 0.015) (Fig. 5 b). No correlation was found between calcium in urine and other bone metabolic markers.

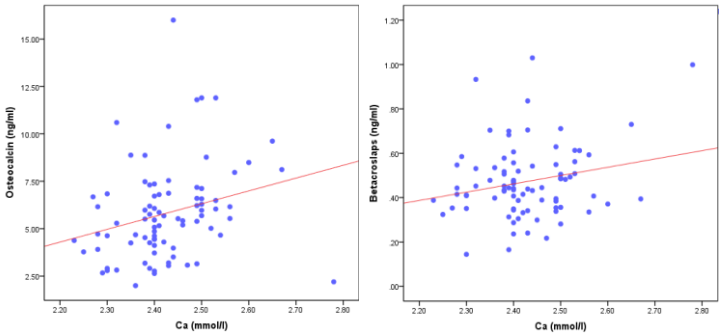

Figure 4 a, b). Correlation of total serum Ca with osteocalcin and Beta Crosslaps

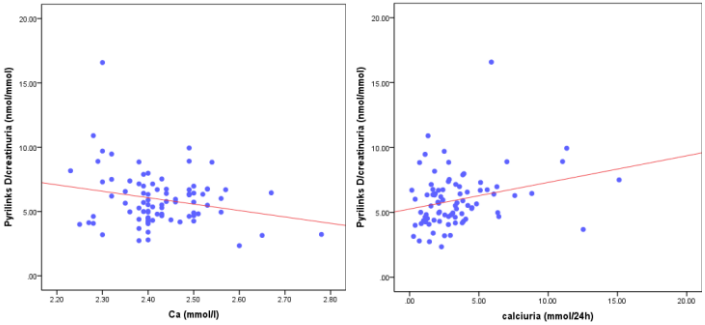

Figure 5 a, b). Correlation of DPD/Cr ratio with total serum and urine Ca

Subsequent regression analyses revealed that total serum Ca was positively associated with OC (β = 0.248; p = 0.042) and negatively with DPD/Cr (β = -0.268; p = 0.012). No dependence was found with Beta Crosslaps. No

dependences between Ca^{2+} and bone turnover were found from the regression analyses. With urinary calcium, a positive dependence was found only with DPD/Cr ($\beta = 0.272$; $p = 0.014$).

Because of the relationship between glomerular filtration and calcium metabolism, we performed an additional correlation analysis. The association between BMD and Ca^{2+} remained significant after eGFR adjustment. According to the bone metabolic markers and Ca levels, we found that only the relationship between total serum Ca and OC remained significant ($r = 0.242$; $p = 0.027$) after eGFR adjustment.

However, the analysis of the relationship between fracture risks and total serum Ca levels indicated positive associations with the 10-year risk of MOF ($r = 0.296$, $p = 0.006$) and HF ($r = 0.308$, $p = 0.004$), which persisted after eGFR adjustment. On the other hand, additional analysis revealed that thiazide intake, albeit insignificant, was associated with a reduction in fracture risk. No dependences with fracture risk were found for Ca^{2+} and calcium in urine, even after considering thiazide intake.

Phosphate metabolism: The analysis revealed a positive dependence of serum P with BMD in the studied skeletal areas (for BMD $r = 0.422$; $p < 0.001$) (Fig. 6), independent of eGFR. On the other hand, a positive relationship was found between phosphaturia with BMD in the femoral region (for BMD Femoral Neck $r = 0.246$, $p = 0.024$; for BMD Total Neck $r = 0.213$, $p = 0.05$), but it was eGFR dependent.

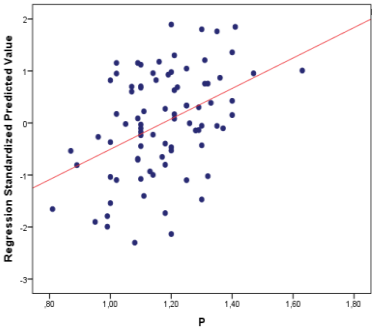

Figure 6. Dependence of BMD (g/cm^2) and serum P(mmol/l)

We found no association between serum P levels and phosphaturia with the bone metabolic markers. We found no relationship between serum P levels and the fracture risks. On the other hand, there was a negative relationship between phosphaturia and the 10-year risk of MOF ($r = -0.247$; $p = 0.004$), but it was eGFR dependent.

Parathyroid hormone: We did not find a dependence of PTH with T score and BMD in the considered skeletal regions, with the bone metabolic markers and with the fracture risks. A positive correlation was found between PTH and Ca^{2+} ($r = 0.310$, $p = 0.005$), regardless of thiazide intake. No dependence was found with total serum Ca and calcium in urine. There was a negative correlation between PTH and serum P levels ($r = -0.246$, $p = 0.024$). No dependence was found on phosphaturia.

Vitamin D: No significant difference in 25(OH)D levels was found between the analyzed according to BMD groups. However, the analysis of 25(OH)D revealed that the total group was dominated by cases of deficiency and insufficiency of Vitamin D in all three analyzed according to BMD groups (Fig. 7).

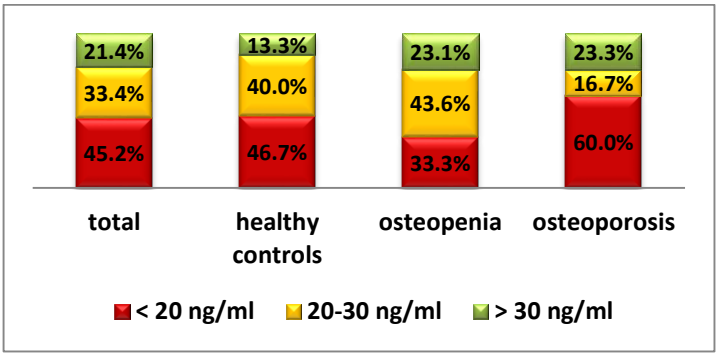

Figure 7. Distribution according to the levels of 25(OH)D

With predominant vitamin D deficiency and insufficiency, no significant differences and dependencies with the bone density indices were found. However, additional analysis revealed that the serum levels of 25(OH)D correlated positively with BMD in healthy controls ($r = 0.994$; $p < 0.001$), while other factors were found in the patients with osteopenia and osteoporosis.

No significant differences and dependences of the level of vitamin D with the bone metabolic markers were found in the whole group and in the analyzed according to BMD groups. The 10-year risk of MOF and HF also indicated no correlation with 25(OH)D levels. Furthermore, no dependence was found with PTH, total serum Ca, Ca^{2+}, calcium in urine, serum P and phosphaturia.

Discussion: A number of researchers highlight the rapid bone loss after the onset of MP (Jilka 1992, Garnero 1996), which presage the development of osteoporosis. The results of analyses in our study indicated that in postmenopausal age, the higher levels of OC and Beta Crosslaps were associated with lower BMD and higher fracture risks. The established negative correlations between BMD in different skeletal regions with OC and Beta Crosslaps suggest that the reduction of BMD in postmenopausal age is associated with a higher level of bone turnover. These data are consistent with the results of Garnero et al. (1996). They found that the main factor determining the development of osteoporosis in postmenopausal age was increased bone metabolism. The authors commented that because of estrogen deficiency in this period the degree of bone formation increases by 37-52%, while the degree of bone resorption increases by 79-97%. Increased bone resorption in the onset of ovarian failure ultimately leads to bone loss, as the rate of bone formation cannot compensate bone resorption (Fujiwara 2016, Xiong 2015, Udagawa 1999). The lack of established significant correlations between DPD/Cr with BMD and fracture risks, as well as the lack of association with the other bone metabolic markers in our study were associated with the additional influence of some extraosseous factors affecting glomerular filtration.

The analyses of calcium metabolism in our study suggested that maintaining a positive calcium balance is associated with higher bone mass in postmenopausal women, as higher, but within the reference ranges, biologically active calcium levels (Ca^{2+}) in the body and less loss of urinary Ca were associated with higher BMD in the studied skeletal regions. On the other hand, we found that higher, but within the reference ranges, total serum Ca levels were associated with higher fracture risks, which we associated with the replenishment of the circulating calcium depot by increased bone metabolism. However, according to our analyses, the net effect of the total serum Ca on the bone metabolism was associated with increased bone

formation. It should be added that the renal function and the intake of drugs that affect the Ca excretion could reflect the relationship between the calcium metabolism and BMD, bone metabolic parameters and fracture risks. Regarding **phosphate metabolism**, we found a positive correlation between the levels of serum and urinary P with BMD and a negative correlation between the urinary P levels and the 10-year risk of MOF. According to these results and because of the dependence between urinary and serum P, we suggested that the lower urinary excretion of P in the case of preserved glomerular filtration was determined by its lower intake, which could adversely affect bone health. The established positive correlation of PTH with Ca^{2+} and negative dependence of PTH with serum P correspond to the physiological effects of the hormone on Ca-P metabolism. On the other hand, the analysis of vitamin D revealed that 78.6% of the women in this study was with 25(OH)D levels below the desired values. These results were similar to the epidemiological data published in 2016 for the Bulgarian population, which indicated that 75.8% of our population was in a state of deficiency or insufficiency of vitamin D (Borisova 2016). However, the positive correlation between 25(OH)D and BMD that we found in healthy controls supports the role of the vitamin in maintaining normal bone mass, as well as the importance of compensating its low levels for the prevention of osteoporosis (Nuti 2019).

4.2. Evaluation and analysis of the relationship between age and duration of menopause and bone health

The women with proven osteoporosis in the study were older and have a longer duration of MP, both compared to healthy controls and compared to women with osteopenia (Table 1). In addition, we reported that about 70% of the changes in bone density were associated with the long duration of MP and the advanced age of the women. We defined as a threshold value of the age above which the risk of osteoporosis increased was 62.5 years (AUC = 0.647 (0.518-0.775); $p = 0.027$) with a sensitivity of 56.7% and a specificity of 70.4% (Figure 8a). We defined as a threshold value of the duration of MP, above which the risk of osteoporosis increases 12.5 years (AUC = 0.738 (0.616-0.860); $p < 0.001$) with a sensitivity of 63.3% and a specificity of 72.2% (Fig. 8 b).

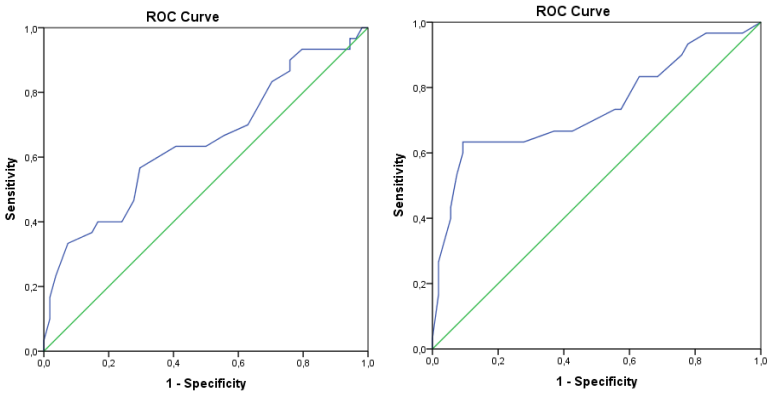

Figure 8 a) and b). ROC curve analysis to determine the threshold age and duration of menopause

Bone density according to age and duration of menopause

Some differences were found from the correlations between age and duration of MP with the parameters of BMD in the analyzed skeletal regions (Table 3).

Table 3. Correlation of BMD with age and duration of menopause (r correlation; p value)

Skeletal region	Age	Duration of menopause
	BMD (g/cm^2)	
L1	**r = -0.268; p = 0.014**	**r = -0.352; p = 0.001**
L2	**r = -0.260; p = 0.017**	**r = -0.373; p < 0.001**
L3	**r = -0.322; p = 0.003**	**r = -0.420; p < 0.001**
L4	r = -0.101; p = 0.359	r = -0.193; p = 0.078
L1-L4	**r = -0.227; p = 0.038**	**r = -0.332; p = 0.002**
Femoral Neck	**r = -0.239; p = 0.029**	**r = -0.361; p = 0.001**
Upper Neck	**r = -0.225; p = 0.004**	**r = -0.371; p = 0.001**
Lower Neck	**r = -0.292; p = 0.007**	**r = -0.406; p < 0.001**
Ward's triangle	**r = -0.291; p = 0.007**	**r = -0.409; p < 0.001**
Trochanter	r = -0.036; p = 0.743	r = -0.169; p = 0.123
Shaft	r = -0.052; p = 0.639	r = -0.084; p = 0.445
Total Neck	r = -0.145; p = 0.187	**r = -0.260; p = 0.017**

The results of the analyses revealed that the age did not correlate with BMD at the levels of L4, Trochanter, Shaft and Total Neck. A weak to moderate inverse correlation was established in the other skeletal regions. These dependencies remained significant after considering the anthropometric parameters. Regarding the duration of MP, no significant relationship was found with BMD at the levels of L4 and Trochanter. In the area of Total Neck, a weak to moderate inverse dependence was found, while in the other skeletal regions an inverse moderate relationship was observed. These dependencies also remained significant after considering the anthropometric parameters.

Bone markers according to age and duration of menopause

In a comparative analysis between the duration of MP and the levels of the bone metabolic markers, it was found that women with a duration of MP up to 10 years were with higher levels of OC than women with a duration of MP over 10 years (6.50 ± 2.78 ng/ml to 5.32 ± 2.16 ng/ml; $p = 0.033$). The analysis of Beta Crosslaps and DPD/Cr did not reveal significant differences with the duration of MP. No dependences were found from the initial correlation analyses between the age and the duration of MP with the bone metabolic markers. After eGFR adjustment, we found a positive correlation between the age and DPD/Cr ($r = 0.230$, $p = 0.036$). The correlation analyses, adjusted to BW, BMI and WC, did not reveal changes in the initially established dependencies.

Fractured risk according to age and duration of menopause

The analysis of the 10-year risk of MOF revealed positive strong correlations with age ($r = 0.579$; $p < 0.001$) and duration of MP ($r = 0.558$; $p < 0.001$) (Fig. 9 a). Similar dependences were found for the 10-year risk of HF with age ($r = 0.398$; $p < 0.001$) and duration of MP ($r = 0.439$; $p < 0.001$) (Fig. 9 b). The correlations remained significant after adjustment to BW, BMI and WC.

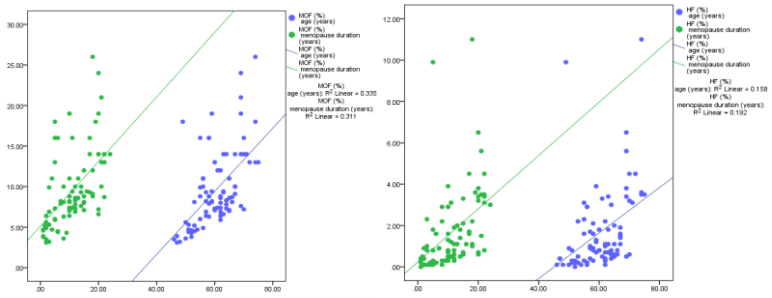

Figure 9 a) and b). Correlation of age and duration of menopause with fracture risks

Discussion: It is well known that the age and the duration of MP are the leading unmodified factors for reduction of BMD, development of osteoporosis and increased fracture risk in postmenopausal women (Borisova 2013). Our results indicated that the changes in BMD were associated in about 70% with the longer duration of MP and with the older age of the women. The data obtained from the correlation analysis of the duration of MP and BMD parameters in the lumbar region we associated with the loss of ovarian function and estrogen deficiency-related changes in the trabecular compartment, which occur at a faster rate. There is also a decrease in BMD at the level of cortical compartment in estrogen deficiency state, but the changes occur at a slower rate and progress with old age (Borisova 2019). We assumed that the development of senile changes involving the cortical compartment of the bone in the current study was not obvious, as the subjects were up to 75 years old. In this regard, we probably did not find a correlation between BMD and age at the level of proximal femur. However, we reported a positive relationship between both age and duration of MP with the fracture risks.

Regarding bone metabolism, we detected some additional negative relationships. According to the established positive relationship between age and DPD/Cr, we assumed that the bone resorption remains increased with age. On the other hand, we assumed that the bone formation decreases with a longer duration of MP, as women with a duration of MP over 10 years in the observed study were with significantly lower levels of OC. These results suggest an

additional reduction in BMD in the late postmenopausal period because of declining bone formation but the bone resorption sustained increased.

4.3 Evaluation and analysis of the relationship between anthropometric parameters and bone health

A significant difference in BW, but not in WC and BMI was found between the analyzed according to BMD groups (Table 1). It should be noted that 82% of the observed subjects were with WC > 80 cm, but these were all healthy controls, 77% of the cases with osteopenia and 80% of the subjects with osteoporosis. According to the calculated BMI, 31% of the women in this study were normal weight (BMI <25 kg/m^2), and 50% were overweight (BMI 25-30 kg/m^2). The cases with obesity (BMI >30 kg/m^2) were 20% of the total group but only 2 of the women were with second degree obesity and 1 person was with third degree obesity.

From the distribution of women according to BMI in the analyzed according to BMD groups, we reported that in the group of healthy controls the lowest proportion of cases with normal weight was observed. In the osteoporosis group we report the least cases of obesity (Fig. 10).

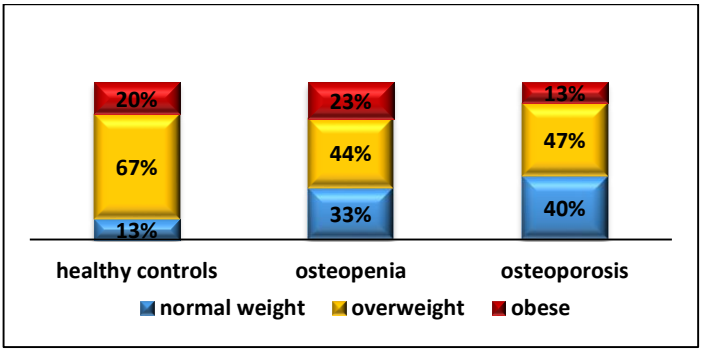

Figure 10. Distribution according to BMI (kg/m^2) in the analyzed according to the BMI groups

Bone density according to the analyzed anthropometric parameters

From the comparative analysis of BMD at the level of L1-L4, Femoral Neck and Total Neck according to WC below and above 80 cm in the total group it

was found that women with WC > 80 cm had significantly higher bone density in the considered skeletal areas (Fig. 11). The results of the comparison of BMD at the level of L1-L4, Femoral Neck and Total Neck according to BMI also revealed a significant difference as women with normal weight were with significantly lower bone density compared to those with overweight and obesity (Fig. 12).

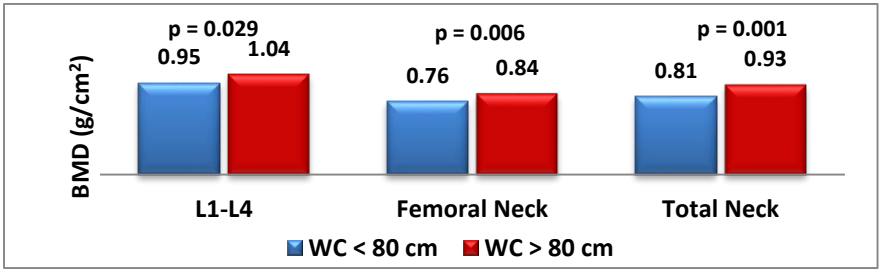

Figure 11. Comparative analysis of BMD according to waist circumference

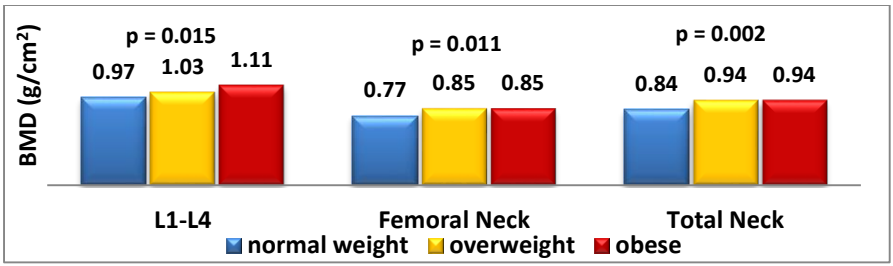

Figure 12. Comparative analysis of BMD according to BMI (kg/m^2)

In addition, the significant difference in BW between the analyzed according to BMD groups revealed that women with osteoporosis were with the lowest BW (65.43 ± 12.08 kg). As a threshold value of BW, below which the risk of osteoporosis increases, we determined 66.5 kg (AUC = 0.325 (0.203-0.446); p = 0.008) with a sensitivity of 36.7% and a specificity of 37.0% (Fig. 13). With BW below this threshold were 46% of the subjects in the total group, but 63% of the cases with osteoporosis, 46% of the women with osteopenia and only 13% of the healthy controls.

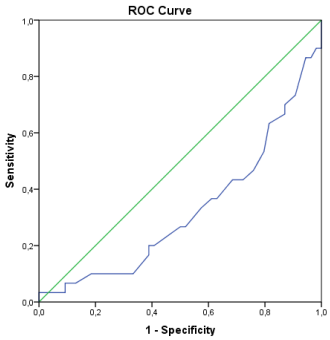

Figure 13. ROC curve analysis to determine the threshold value of BW in women with osteoporosis

From the comparative analysis of BMD at the level of L1-L4, Femoral Neck and Total Neck according to BW below and above 66.5 kg in the total group it was found that women with higher BW were with significantly higher bone density in the considered skeletal areas (Fig. 14).

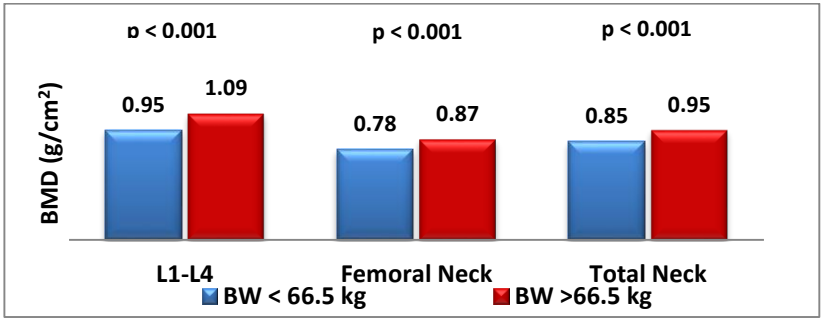

Figure 14. Comparative analysis of BMD according to body weight

The results of the subsequent correlation analyses between WC, BMI and BW with BMD in the considered skeletal regions revealed that there was a positive relationship between these anthropometric parameters and bone density (Table 4). After adjustment to age and duration of MP the positive correlations between these anthropometric parameters and BMD remained significant. Despite the established positive correlations between WC, BMI and BW with

BMD, the subsequent partial correlation analyses did not suggest a synergistic or an additive effect of the three anthropometric parameters on bone density (Table 4). After BW adjustment a loss of initially detected positive dependences of WC and BMI with BMD was reported. Moreover, a negative correlation was found between BMD at the Femoral Neck level and BMI after BW adjustment. On the other hand, the positive relationship between BW and BMD remained significant after correcting the correlation analysis according to WC and BMI. These results suggest that the effects of visceral adipose tissue on the skeleton are mediated by mechanical loading of the skeleton but not by its metabolic effects.

Table 4. Adjusted dependence of BMD with anthropometric parameters (r correlation; p value)

	WC (cm)	**BMI (kg/m^2)**	**BW (kg)**
BMD L1-L4 (g/cm^2)	**r = 0.264; p = 0.015**	**r = 0.295; p = 0.006**	**r = 0.446; p < 0.001**
BMI adjusted	r = 0.060; p = 0.588	-	**r = 0.388; p < 0.001**
WC adjusted	-	r = 0.150; p = 0.176	**r = 0.418; p < 0.001**
BW adjusted	r = - 0.203; p = 0.066	r = -0.179; p = 0.106	-
BMD Femoral Neck (g/cm^2)	**r = 0.338; p = 0.002**	**r = 0.223; p = 0.042**	**r = 0.409; p < 0.001**
BMI adjusted	**r = 0.268; p = 0.014**	-	**r = 0.428; p < 0.001**
WC adjusted	-	r = - 0.062; p = 0.578	**r = 0.243; p = 0.027**
BW adjusted	r = 0.004; p = 0.969	**r = -0.260; p = 0.018**	-
BMD Total Neck (g/cm^2)	**r = 0.393; p < 0.001**	**r = 0.330; p = 0.002**	**r = 0.457; p < 0.001**
BMI adjusted	**r = 0.230; p = 0.037**	-	**r = 0.355; p = 0.001**
WC adjusted	-	r = 0.049; p = 0.663	**r = 0.256; p = 0.019**
BW adjusted	r = 0.033; p = 0.767	r = -0.125; p = 0.261	-

Bone metabolic markers according to the analyzed anthropometric parameters

From the comparison of the bone metabolic markers according to WC below and above 80 cm in the total group, only a significantly higher level of DPD/Cr was found at higher WC (6.23 ± 2.14 nmol/mmol to 4.65 ± 1.39 nmol/mmol,

p = 0.008). However, in the osteoporosis group, there was a significantly lower level of Beta Crosslaps at WC above 80 cm (0.45 ± 1.12 ng/ml to 0.60 ± 0.28 ng/ml, p = 0.047) but an insignificant trend to a lower level of OC (6.05 ± 2.41 ng/ml to 8.16 ± 4.40 ng/ml, p> 0.05). Subsequent correlation analysis between WC and bone metabolic markers in the total group revealed an inverse relationship with OC (r = -0.217; p = 0.048). A similar trend was found for the relationship between WC and Beta Crosslaps (r = -0.226; p = 0.039), while with DPD/Cr a positive weak association was found (r = 0.277; p = 0.011) (Fig. 15 a, b, c).

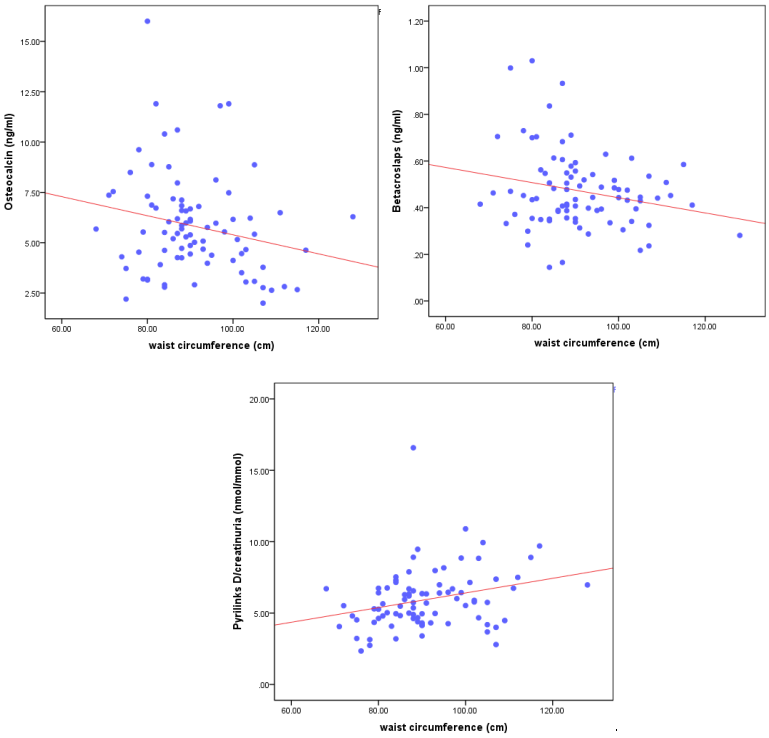

Figure 15 a), b) and c). Correlation of waist circumference with osteocalcin, Beta Crosslaps and DPD/Cr ratio

From the comparison of the bone metabolic markers according to BMI in the total group, only a significant difference in DPD/Cr levels was found (5.13 ± 1.26 nmol/mmol for normal weight; 6.32 ± 2.50 nmol/mmol for overweight; 6.39 ± 1.89 nmol/mmol for obesity; p = 0.046), which indicated that the increase in BMI increased the level of the urinary marker of bone resorption. When comparing the bone metabolic markers according to BMI in the analyzed according to BMD group no additional significant differences were found, but there was a trend to decrease the levels of OC and Beta Crosslaps with increasing BMI in osteoporosis group (Fig. 16 a, b). Subsequent correlation analysis did not reveal a relationship between BMI with OC and Beta Crosslaps, but a significant increase in DPD/Cr levels (r = 0.249; p = 0.022) with an increase in BMI was found.

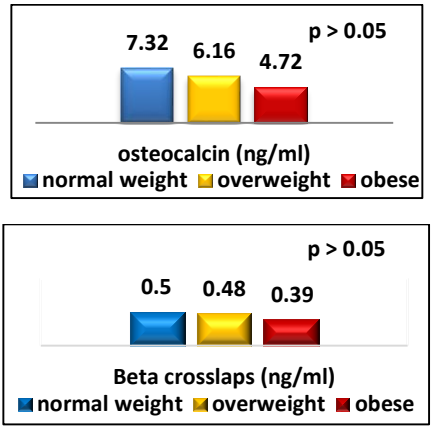

Figure 16 a) and b). Comparative analysis of the mean values of OC and Beta Crosslaps according to BMI (kg/m^2) in the group of osteoporosis

From the comparison of the bone metabolic markers according to the determined threshold values of BW, no differences were found in the total group and in the analyzed according to the BMD groups. On the other hand, the correlations of BW with the bone metabolic markers followed the trend of established relationships in BMI. No dependence was found with OC and Beta Crosslaps, while a positive relationship was found with DPD/Cr (r = 0.263; p = 0.015).

Because of opposite associations between the anthropometric parameters with the markers of bone resorption (Beta Crosslaps and DPD/Cr), additional analyses were performed. We found strong positive dependences of eGFR with WC ($r = 0.593$; $p < 0.001$), BMI ($r = 0.550$; $p < 0.001$) and BW ($r = 0.709$; $p < 0.001$). These results suggest that higher anthropometric values are associated with higher glomerular filtration. On the other hand, the weak positive correlations of DPD/Cr with WC, BMI and BW became insignificant after eGFR adjustment. Subsequent regression analysis between the anthropometric parameters and the bone metabolic markers revealed that the levels of OC were negatively associated with WC ($r = -0.217$; $p = 0.024$) and BMI ($r = -0.184$; $p = 0.047$), the levels of Beta Crosslaps were negatively related to WC ($r = -0.226$; $p = 0.019$), and between DPD/Cr and the three anthropometric parameters a positive relationship remained, but it became insignificant after eGFR adjustment. To determine the independent significance of the considered anthropometric indicators in relation to the bone metabolic markers, we conducted a multifactorial stepwise analysis. Higher WC was distinguished as a significant negative predictor of bone turnover, as it was associated with lower levels of OC ($\beta = -0.217$; $p = 0.048$) and Beta Crosslaps ($\beta = -0.226$; $p = 0.039$). Additional calculations indicated that 31% of bone marker levels depend on WC. The positive relationship between WC and DPD/Cr ($\beta = 0.277$; $p = 0.011$) was determined by eGFR. The established dependencies did not change after taking into account the age and duration of MP.

Fracture risk according to the analyzed anthropometric indicators

From the comparative analysis of the fracture risks according to WC and BMI no significant differences were found. No correlations were found with the fracture risks. However, from the comparative analysis of the fracture risks according to the determined threshold values of BW, significant differences were found (Fig. 17). In addition, a negative correlation was found between BW and the fracture risks. The relationship between the 10-year risk of MOF and BW was weak to moderate ($r = -0.275$; $p = 0.011$), and between the 10-year risk of HF and BW was moderate ($r = -0.353$; $p = 0.001$).

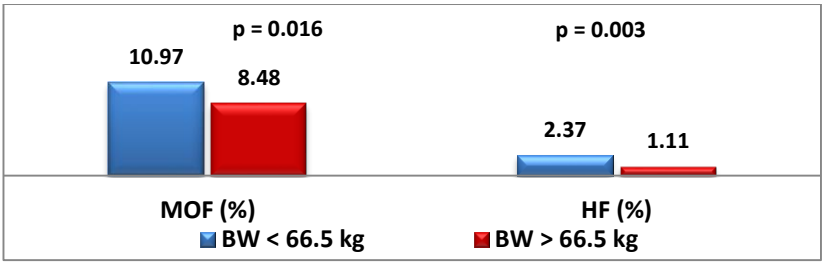

Figure 17. Comparative analysis of fracture risks according to body weight

Subsequent regression analysis confirmed that BW was negatively associated with a 10-year risk of MOF ($r = -0.275$, $p = 0.006$) and HF ($r = -0.357$, $p < 0.001$). However, with BMI, regression analysis revealed a weak negative correlation with the 10-year risk of HF ($r = -0.184$, $p = 0.047$). Marginal negative dependence was also found between WC and the 10-year risk of HF ($r = -0.178$, $p = 0.053$).

To determine the independent significance of the considered anthropometric parameters in relation to the fracture risks, we conducted a multifactorial stepwise analysis. Higher BW was noted as a significant negative predictor of 10-year risk of MOF ($\beta = -0.730$, $p < 0.001$) and HF ($\beta = -0.730$, $p < 0.001$), but a positive association was found for BMI as with a 10-year risk of MOF ($\beta = 0.532$, $p = 0.009$) and HF ($\beta = 0.441$, $p = 0.026$). After considering age and duration of MP, the negative associations of BW with the 10-year risk of MOF ($\beta = -0.246$, $p = 0.006$) and HF ($\beta = -0.299$, $p = 0.002$) remained significant. Additional BW threshold values were found below which the fracture risks increased. For the 10-year risk of MOF the cut-off was 62 kg (AUC = 0.856 (0.729-0.982); $p = 0.037$) with a sensitivity of 72.8% and a specificity of 100%, and for the 10-year risk of HF the cut-off was 66.5 kg. (AUC = 0.617 (0.466-0.767); $p = 0.031$) with a sensitivity of 59.1% and a specificity of 66.7%. Below these BW threshold values, it could be said that the probability of fracture increases.

Vitamin D and anthropometric indicators. From the comparative analysis of the 25(OH)D levels according to WC we found a trend to lower vitamin D levels at WC > 80 cm (21.39 ± 8.09 ng/ml to 24.98 ± 13.03 ng/ml, $p > 0.05$), but a significant negative association was found from the correlation analysis

(r = -0.228; p = 0.037). From the comparative analysis of the 25(OH)D levels according to BMI, we found a significant difference (p = 0.044), which indicated a significant decrease in vitamin D levels with increasing BMI. Moreover, there was a pronounced deficiency of Vitamin D in obese women (Fig. 18).

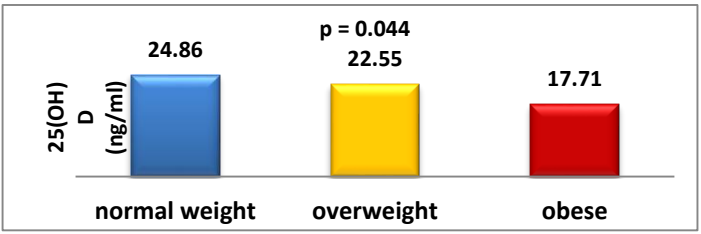

Figure 18. Mean values of 25(OH)D according to BMI (kg/m^2)

Subsequent correlation analysis demonstrated that there was a negative relationship between vitamin D levels and BMI (r = -0.248; p = 0.023).

When comparing the 25(OH)D levels according to the determined threshold values of BW, no differences were found. We did not find also correlations between the two parameters. On the other hand, a positive relationship (r = 0.219; p = 0.046) was found between WC and PTH levels. The strength of this association increased after eGFR adjustment (r = 0.399; p <0.001). Subsequent multifactorial stepwise analysis revealed that the PTH levels were positively associated with WC (β = 0.584, p <0.001), regardless of age, duration of MP, other anthropometric parameters, Ca-P metabolic parameters and eGFR. On the other hand, the 25(OH)D level remained negatively related to BMI (β = -0.248, p = 0.023) after considering these variables.

Discussion: It has been suggested that visceral adipose tissue may have a beneficial effect on bone integrity by stimulating osteoblast activity and inhibiting bone resorption through adipocyte-related hormones and growth factors (Reid 2008). Because of initially established positive correlations of WC and BMI with BMD as well as negative dependences from the regression analysis of the two anthropometric parameters with the fracture risks, we also supposed that abdominal obesity could be positively associated with postmenopausal bone health. However, the results of the subsequent adjusted

analyses suggested that the higher BW was the anthropometric parameter, which was associated with better BMD indices and with lower fracture risks in obesity. This supposition was supported by the loss of the positive relationship between WC and BMI with the parameters of BMD after BW adjustment. In addition, after multifactorial stepwise analysis, we found a positive relationship between BMI and fracture risks and no negative association of WC with fracture risks. It should be noted that these dependencies were observed in women with a predominance of overweight and first degree obesity, so we could not exclude the possibility of a change in the strength or direction of the relationships in extreme obesity.

Based on the established associations of the bone metabolic markers with the anthropometric parameters and after additional analyses similar to other authors (Sharma 2020), we found that the accumulation of visceral adipose tissue was associated with lower levels of bone metabolism because of the negative correlations between WC with OC and Beta Crosslaps. On the other hand, we assumed that the levels of the bone resorption marker DPD/Cr are determined not only by the level of bone metabolism, but also by additional extraosseous factors that should be taken into account when interpreting the results. For example, we found relationships between the anthropometric parameters and the renal function, which suggested that higher levels of DPD/Cr may be associated with higher glomerular filtration, which we observed in overweight and obese women.

The lack of positive correlation of BMD with WC and BMI after BW-adjusted analysis, the established lower level of bone turnover in obesity and the positive dependence of the fracture risks with BMI from the multifactorial stepwise analysis suggested that obesity could counteract the positive relationship of postmenopausal bone health with BW and related mechanical skeletal loading. It was further commented in the literature that abdominal obesity could have a negative effect on bone microarchitecture, despite normal BMD values measured with DEXA (Migliaccio 2013, Caffareli 2014). These adverse effects could be explained by the adverse metabolic abnormalities that are associated with visceral adipose tissue.

The lower levels of 25(OH)D found in the present study in postmenopausal women with obesity were consistent with the results of a number of studies

(Walsh 2018, Sharma 2020). Vitamin D deficiency/insufficiency, which is observed during the accumulation of visceral adipose tissue has also been commented as an additional factor in deteriorating bone health in obesity. As other authors, we similarly found higher levels of PTH in obesity with lower serum concentrations of 25(OH)D (Sharma 2020). However, we reported a lower level of bone turnover. The established negative association between WC and Beta Crosslaps in postmenopausal women, despite the found positive dependence of WC with PTH, suggested the presence of additional factors that play a role in determining bone resorption in these situation. On the other hand, the negative association of WC with OC does not guarantee that the lower bone turnover is combined with a decrease in bone mineralization. On the contrary, it is assumed that with a higher amount of visceral adipose tissue the bone matrix may become hypermineralized, which is associated with poor bone microarchitecture, explaining the higher risk of fractures in obese people, despite normal or higher BMD compared to weaker individuals. In addition, lower bone turnover in obesity could lead to the accumulation of microdamages as a result of daily repeated loads on the skeleton, which further leads to lower bone quality (Sharma 2020).

However, the mechanical load on the skeleton associated with higher BW is important for maintaining bone health. On the other hand, unbalanced weight loss in obesity may adversely affect bones. Accordingly, the results of a meta-analysis of randomized controlled trials revealed that BMD at the level of proximal femur decreased significantly with BW loss after 4 months, and the reduction in BMD at the level of lumbar spine was significant after 13 months. In comparison, weight loss in combination with exercise does not lead to a reduction in BMD (Soltani 2016). Therefore, the combination of a balanced diet with restriction of caloric intake, but sufficient calcium and vitamin D intake and increased physical activity could contribute to the reduction of adipose tissue, while maintaining muscle and bone mass.

4.4. Evaluation and analysis of the relationship between carbohydrate metabolism and bone health

With fasting BG ≥ 5.6 mmol/l were 51% of the subjects, but in the group of osteopenia the proportion was the highest (56%). With BG at 120 min ≥ 7.8 mmol/l were 13% of the women, but they were distributed among the group of

osteopenia and osteoporosis. With HOMA index > 2 were 46% of the observed cases but the highest proportion is among healthy controls (60%).

Bone density according to the parameters of carbohydrate metabolism

No significant differences were found from the comparative analysis of T score and BMD in the examined skeletal regions according to the fasting BG below and above 5.6 mmol/l and according to the BG at 120 min below and above 7.8 mmol/l. No dependences were found from the initial correlation analysis of BMD with BG at 0 and 120 min. On the other hand, the comparative analysis of BMD according to HOMA index below and above 2 revealed significantly higher values of BMD in the area of the proximal femur at index values above 2 (Table 5). In addition, positive correlations were found between BMD at the proximal femur level and fasting insulin (with BMD Femoral Neck r = 0.204, p = 0.031, with BMD Total Neck r = 0.277, p = 0.011). Similar dependences were found for the HOMA index (with BMD Femoral Neck r = 0.201, p = 0.034, with BMD Total Neck r = 0.283, p = 0.009).

Table 5. Comparative analysis of BMD according to HOMA index (mean ± SD)

Skeletal region	**T score (±SD)**			**BMD (g/cm^2)**		
	HOMA index <2	**HOMA index >2**	**P value**	**HOMA index <2**	**HOMA index >2**	**P value**
L1	-1.72±1.15	-1.27±1.06	0.07	0.92±0.14	0.98±0.13	0.76
L2	-1.89±1.22	-1.36±1.42	0.069	0.97±0.14	1.04±0.17	0.70
L3	-1.26±1.34	-0.82±1.48	0.154	1.05±0.16	1.10±0.18	0.165
L4	-1.32±1.59	-0.87±1.47	0.191	1.04±0.19	1.09±0.18	0.212
L1-L4	-1.42±1.20	-1.04±1.30	0.160	1.00±0.15	1.06±0.15	0.106
Femoral Neck	-1.69±0.68	-1.37±0.91	0.075	0.81±0.10	0.85±0.13	0.177
Upper Neck	-1.51±0.82	-1.31±1.04	0.339	0.64±0.10	0.66±0.13	0.328
Lower Neck	-	-	-	0.96±0.10	1.02±0.14	**0.021**
Ward‘s triangle	-2.16±0.79	-1.89±1.03	0.191	0.63±0.10	0.66±0.13	0.187
Trochanter	-1.07±0.96	-0.69±0.98	0.084	0.73±0.11	0.77±0.11	0.081
Shaft	-	-	-	1.03±0.21	1.12±0.17	**0.033**
Total Neck	-1.02±0.87	-0.57±1.03	**0.032**	0.88±0.11	0.94±0.13	**0.032**

When comparing the correlations of the parameters of carbohydrate metabolism with BMD in the femoral region between the analyzed according to BMD groups, it was found that the positive relationship of fasting insulin

and HOMA index with bone indices remained significant only in the group of healthy controls (fasting insulin with BMD Femoral Neck r = 0.525, p = 0.044; with BMD Total Neck r = 0.531, p = 0.042; for HOMA index with BMD Femoral Neck r = 0.594, p = 0.02; with BMD Total Neck r = 0.598, p = 0.019). We assumed that the loss of correlation in the groups of osteoporosis and osteopenia was related to the influence of age, duration of MP and / or anthropometric parameters. In this regard, we conducted additional correlation analyses. When recalculating the correlations between BMD and the estimated parameters of carbohydrate metabolism according to age and duration of MP in the total group, no significant changes were found in the initially established positive relationships with fasting insulin and HOMA index. When recalculating the correlations between BMD and the estimated parameters of carbohydrate metabolism according to WC and BMI in the total group, loss of initially positive association with fasting insulin and NOMA index was observed. Additionally, a negative correlation was found between BMD at the Femoral Neck level and BG at 120 min (r = -0.236, p = 0.033). From the correlation analysis of BMD and the estimated parameters of carbohydrate metabolism according to BW, no significant dependences were found with the levels of BG, insulin and HOMA index.

On the other hand, when recalculating the correlation of BMD with BG and insulin compared to HOMA index, additional dependences were found. Loss of a positive relationship between fasting insulin and BMD was observed at the level of proximal femur, but a negative correlation between an insulin at 120 min and BMD at the Femoral Neck level was found (r = -0.262, p = 0.017). Figure 19 shows the loss of positive correlation at HOMA index > 2 between fasting insulin and BMD at the Femoral Neck level (r = 0.122, p > 0.05) and the presence of a significant negative correlation of an insulin at 120 min with BMD at the Femoral Neck level (r = -0.268, p <0.05).

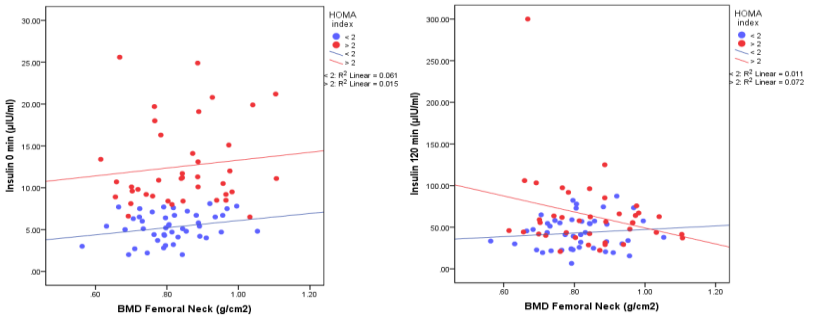

Figure 19. Correlation between BMD at the Femoral Neck level and insulin according to HOMA index

To determine the independent relationship between the components of carbohydrate metabolism and obesity with BMD, we performed a multifactorial stepwise analysis. The obtained results indicated that the established positive relationships of fasting insulin and HOMA index with BMD at the proximal femur level were determined by WC ($\beta = 0.380$, $p < 0.001$). On the other hand, BG at 120 min remained negatively associated with BMD at the Femoral Neck level ($\beta = -0.231$, $p = 0.03$), regardless of HOMA index, WC and BMI. However, when added to the analysis BW, it was found that only BMI was negatively associated with BMD at the Femoral Neck level ($\beta = -0.425$, $p = 0.027$), while BW proved to be the leading positive predictor of better bone density ($\beta = 0.747$, $p < 0.001$).

Bone metabolic markers according to the parameters of carbohydrate metabolism

From the comparative analysis of the bone metabolic markers according to the fasting BG, significant differences were found in the OC levels (Fig. 20), which revealed that individuals with BG below 5.6 mmol/l had higher levels of the bone formation marker. No significant difference was found in the bone metabolic markers according to HOMA index below and above 2 and according to BG at 120 min below and above 7.8 mmol/l.

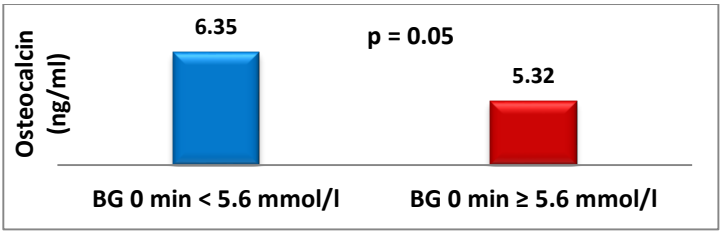

Figure 20. Osteocalcin levels according to fasting blood glucose

When comparing the estimated parameters of carbohydrate metabolism according to the threshold values of the bone metabolic markers, it was found additionally that in subjects with HOMA index below 2 insulin levels at 120 min were significantly lower at OC levels above threshold value ($p = 0.017$), while in women with HOMA index over 2 such a difference was not found (Fig. 21). These results suggest that, while maintaining insulin sensitivity, higher levels of the bone formation marker are associated with lower stimulated insulin levels.

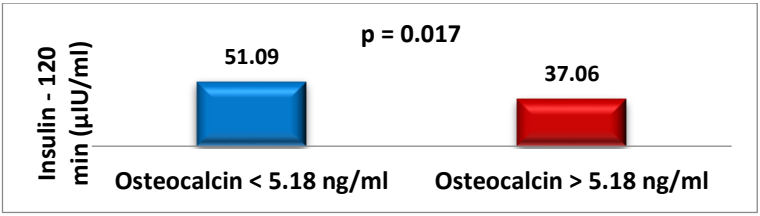

Figure 21. Comparative analysis of insulin levels at HOMA index below 2 according to osteocalcin thresholds

No correlations of OC and Beta Crosslaps with the estimated parameters of carbohydrate metabolism were found. The established positive relationship between DPD/Cr and HOMA index ($r = 0.028$, $p = 0.037$) became insignificant after eGFR adjustment. When recalculating the correlation dependences of the bone metabolic markers with the estimated parameters of carbohydrate metabolism according to anthropometric parameters, no dependence was found.

Fracture risk according to the parameters of carbohydrate metabolism

No significant difference was found in the 10-year risk of MOF and HF according to the BG levels and HOMA index. On the other hand, the correlation analysis between the fracture risks and the parameters of carbohydrate metabolism revealed a positive dependence between the 10-year risk of MOF and BG at 120 min ($r = 0.265$, $p = 0.015$). The dependence between the 10-year risk of HF and BG at 120 min was marginal ($r = 0.205$, $p = 0.061$). After adjusting the analysis to WC and BMI, an additional positive dependence was found between the fasting BG and the 10-year risk of MOF ($r = 0.252$, $p = 0.022$) and HF ($r = 0.260$, $p = 0.018$). On the other hand, the positive correlation of BG at 120 min with the 10-year risk of MOF was preserved ($r = 0.295$, $p = 0.007$), but the association with the 10-year risk of HF became statistically significant ($r = 0.248$, $p = 0.024$). Positive relationships between the fracture risks with the fasting BG and BG at 120 min were also reported after BW adjustment (for fasting BG and MOF $r = 0.258$, $p = 0.019$; for BG at 120 min and MOF $r = 0.278$, $p = 0.011$; for fasting BG and HF $r = 0.266$, $p = 0.015$; for BG at 120 min and HF $r = 0.223$, $p = 0.042$). Additional dependencies were found after correcting the correlation analyses of BG and insulin levels with the fracture risks according to HOMA index. Positive correlations were established between the 10-year risk of MOF and the fasting BG ($r = 0.279$, $p = 0.011$), BG at 120 min ($r = 0.275$, $p = 0.012$) and the insulin at 120 min ($r = 0.219$, $p = 0.047$). Negative dependence was revealed with the fasting insulin ($r = -0.275$, $p = 0.012$). According to the 10-year risk of HF, similar trends were found, but they did not reach statistical significance. Subsequent analysis revealed that the positive correlation remained significant only at HOMA index > 2 for the 10-year risk of MOF with the fasting BG ($r = 0.341$, $p < 0.05$) and BG at 120 min ($r = 0.417$, $p < 0.05$). At HOMA index < 2 the significance of the correlations wass lost (Fig. 22 a, b).

As for the negative correlation between the 10-year risk of MOF and the fasting insulin, it was found that this dependence was statistically significant only at HOMA index < 2 ($r = -0.396$, $p < 0.05$), while at HOMA index > 2 the relationship remained insignificant. On the other hand, the positive correlation between the 10-year risk of MOF and the insulin at 120 min maintained its significance only at HOMA index > 2 ($r = 0.417$, $p < 0.05$), while at HOMA index < 2 the relationship was insignificant (Fig. 23a and b).

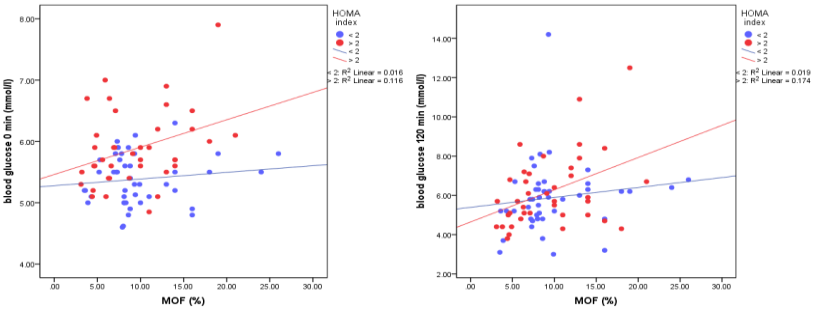

Figure 22 a) and b). Correlation between the 10-year risk of MOF and BG according to the HOMA index

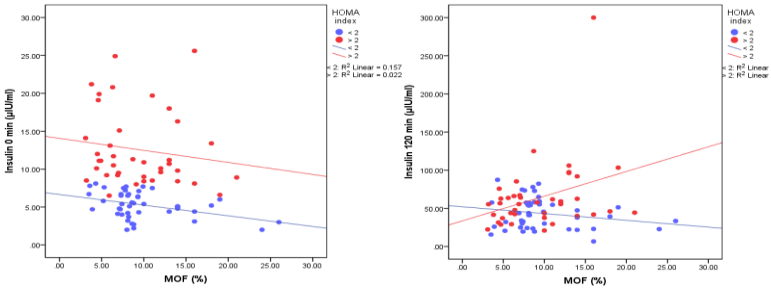

Figure 23 a) and b). Correlation between the 10-year risk of MOF and insulin according to HOMA index

Based on the performed correlation analyses and the obtained results, insulin threshold values for risk assessment were determined. Risk values of fasting insulin for the 10-year risk of MOF were those below 6.15 µIU/ml (AUC = 0.772 (0.446-1.000); p = 0.011) with a sensitivity and specificity of 66.7%. Risk values of insulin at 120 min for the 10-year risk of MOF were those above 39.7 µIU/ml (AUC = 0.720 (0.540-0.900); p = 0.019) with a sensitivity and specificity of 66.7%.

To determine the independent relationship between the components of carbohydrate metabolism and the fracture risks, we performed a multifactorial stepwise analysis. The obtained results revealed that BG at 120 min remained positively associated with the 10-year risk of MOF ($\beta = 0.265$, $p = 0.015$). This dependence persisted unchanged after considering WC and BMI. From the

multifactorial stepwise analysis of the 10-year risk of MOF with the components of carbohydrate metabolism and BW, the positive association with BG at 120 min (β = 0.268, p = 0.011) was again reported, regardless of the negative association with BW (β = -0.278, p = 0.008). In addition, the complex multifactorial stepwise analysis identified fasting BG as positively associated with the 10-year risk of HF (β = 0.210, p = 0.04), independent of the other components of carbohydrate metabolism and the anthropometric parameters.

Vitamin D and carbohydrate metabolism. An additional correlation analysis between the components of carbohydrate metabolism with the levels of vitamin D revealed that there was no relationship between 25(OH)D and the fasting levels of BG and insulin, but there was a negative relationship between 25(OH)D and BG at 120 min (r = -0.225; p = 0.039), as well as with insulin at 20 min (r = -0.288; p = 0.008) (Fig. 24 a, b).

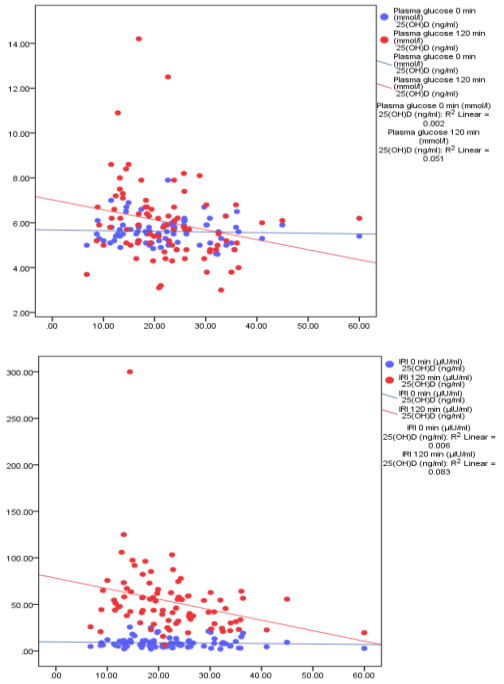

Figure 24 a) and b). Correlation of 25(OH)D with BG and insulin

After WC and BMI adjustment, only a negative relationship between 25(OH)D and insulin levels at 120 min ($r = -0.255$, $p = 0.021$) was preserved.

To determine the independent relationship between the components of carbohydrate metabolism and 25(OH)D levels, we performed a multifactorial stepwise analysis. The results revealed that 25(OH)D remained negatively bound to the insulin at 120 min ($\beta = -0.288$, $p = 0.008$). This relationship remained unchanged after inclusion the anthropometric parameters in the analysis.

Discussion: According to the performed analyses in the present study, several correlations were found between carbohydrate metabolism and BMD in the femoral region, but some of them were modulated by additional factors. From the correlation and regression analyses we find that BG at 120 min was negatively associated with BMD at the femoral neck level, despite that the analyzed women were without T2DM, and a relatively small percentage of them were with impaired glucose tolerance. This relationship remained independent of WC and BMI. On the other hand, it was found that higher basal insulin levels and HOMA index were associated with better BMD parameters at the proximal femur. However, these dependencies were determined by WC and body size. In accordance with our results, Napoli et al. (2019) found that BMD increased with increasing HOMA index. This positive association between IR and BMD in the study also lost its significance after BMI adjustment, which suggests that the relationship was influenced by body size. Another study by Yang et al. (2018) similarly found that IR was associated with higher volumetric cortical BMD, but also with lower bone volume, especially at the cortical compartment among postmenopausal women without T2DM. In addition, the authors found that higher IR was associated with lower bone strength, regardless of the body size. And Campillo-Sánchez et al. (2020) confirmed in their study the negative association of IR with bone quality in postmenopausal women without T2DM as they found BW-independent inverse dependence of TBS with HbA1c, insulin levels and HOMA index. The authors also specified that the direct relationship found between IR and volumetric BMD was determined by the higher BW. Although there was no assessment of bone microarchitecture in the present study, we still found negative correlations between bone integrity and higher insulin levels at 120 min, which we considered to be compensatory hyperinsulinemia because of IR

development. It should be noted that we have not found studies in the literature that compare bone health with insulin levels during OGTT. Our sub-analyses indicated that the positive association between fasting insulin and BMD in the femoral region was lost in IR state. Moreover, a negative association between insulin at 120 min and BMD at the femoral neck was established at HOMA index > 2. These results suggest that the osteoanabolic action of insulin depends on the persistence of systemic insulin sensitivity. On the other hand, the loss of positive correlation between fasting insulin and BMD in the femoral region, as well as the negative correlation between insulin at 120 min and BMD at the femoral neck at HOMA index > 2 raise the question of the loss of insulin sensitivity at the bone level when systemic IR is presented (Wei 2012, Pramojanee 2013). However, we suggested that the relationship between insulin and bone health might be indirectly affected by IR-related oxidative, proinflammatory and lipid changes.

The question remains open in the literature whether the risk of fractures is increased or decreased in state of IR without obvious T2DM. Some researchers have found that people with prediabetes have a lower risk of fractures after considering BMI and/or BMD (de Liefde II 2005, Holmberg 2008). Other researchers have not found a statistically significant difference in the fracture risk among these individuals, although some sub-analysis suggested increased risk (Looker 2016; Napoli 2014). On the other hand, Chen et al. (2020) found that people with prediabetes had a lower prevalence of osteopenia / osteoporosis but a higher prevalence of fractures than people with normal glucose regulation. Skoradal et al. (2018), however, noted that in people with prediabetes aged 55-70 leading a sedentary lifestyle, football training could provide a powerful osteogenic stimulus and improve bone health. This study highlighted the beneficial effects of lifestyle changes on skeletal health in patients with prediabetes. Our results from the analyses between the parameters of carbohydrate metabolism and the fracture risks correspond to the established correlations with BMD in the observed cases, although no dependences with the bone metabolic markers were found. Conducted correlation and adjusted analyses revealed that higher values of fasting BG and BG at 120 min in the absence of T2DM were associated with a higher risk of fractures. However, these relationships could be modified by both WC and body size, as well as the insulin sensitivity state. However, fasting BG

remained independently positively associated with 10-year risk of HF, and BG at 120 min remained independently positively associated with the 10-year risk of MOF. On the other hand, the association of the fasting insulin and the insulin at 120 min with the fracture risks depended on the state of IR. When insulin sensitivity (HOMA index <2) was preserved, higher basal insulin levels were associated with a lower 10-year risk of MOF. On the other hand, basal insulin levels were not associated with reduced fracture risk in IR state. In addition, at IR state (HOMA index > 2), higher stimulated insulin levels (insulin at 120 min) correlated positively with the 10-year risk of MOF.

However, it is discussed that bone might be also a determinant of IR and glucose homeostasis. Pittas et al. (2009) noted, for example, that higher levels of OC were associated with less increase in fasting glucose. Consistent with these results, we found that the levels of bone formation marker were higher at fasting BG below 5.6 mmol/l. It is further commented that OC levels are also associated with insulin sensitivity (Lee 2007, Pittas 2009). The bone formation marker is thought to support insulin secretion through a direct effect on pancreatic β-cells, thus improving insulin sensitivity and significantly alleviating the harmful effects of obesity (Ferron 2008). Accordingly, Pittas et al. (2009) found that OC was negatively associated with the fasting insulin levels and the markers of IR. However, the improvement in insulin homeostasis is not only determined by the ability of the OC to modulate insulin secretion. An additional prerequisite for achieving this effect is the ability of bone to remain sensitive to insulin action when the hormone levels increased (Tonks 2017). In accordance with these data, the lower insulin levels at 120 min when OC was above threshold value at HOMA index < 2 in our study suggested that the bone formation marker could modulate insulin secretion while maintaining sensitivity to the hormone. On the other hand, the lack of such an association in HOMA index > 2 raises the question of the loss of insulin sensitivity at the bone level.

The effect of vitamin D on carbohydrate metabolism is also widely discussed. Its deficiency is thought to play an important role in the development of IR and T2DM, as this affects insulin sensitivity and/or β-cell function (Chiu 2004). We also hypothesized that vitamin D deficiency adversely affects carbohydrate metabolism, as we found negative dependences of BG at 120 min and insulin at 120 min with 25(OH)D levels. In addition, the significance of the

relationship between insulin at 120 min and 25(OH)D levels was maintained despite of WC and body size. In this regard, we hypothesized that vitamin D deficiency, through its effects on carbohydrate metabolism, could affect bone integrity indirectly.

4.5 Assessment and analysis of the relationship between lipid metabolism and bone health

Differences in lipid levels between the analyzed according to BMD groups were not statistically significant (Table 1), even after excluding from the analysis women taking statins, which were 15.5% of the total group (healthy controls 4.8%, osteopenia 8.3%, osteoporosis 2.4%).

Bone and total cholesterol (TC). It was found that in women with osteoporosis the proportion of subjects with TC levels above the upper reference range was higher than in healthy controls and cases of osteopenia (Fig. 25).

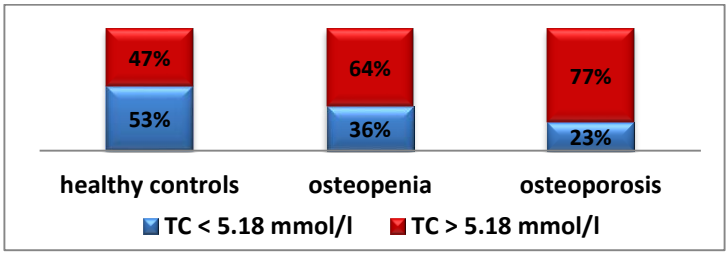

Figure 25. Distribution of total cholesterol between the analyzed according to BMD groups

The comparative analysis between the parameters of bone density according to the levels of TC below and above 5.18 mmol/l in the total group indicated a trend to lower values of T score and BMD in the considered skeletal regions at TC > 5.18 mmol/l, but only at femoral neck difference reached marginal significance (Fig. 26). The same trend, but without statistical significance, was observed after exclusion from the analysis the women taking statins.

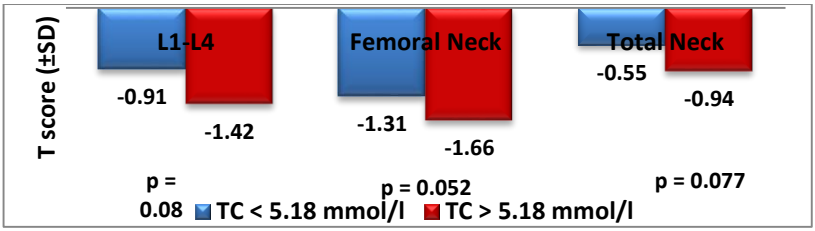

Figure 26. Mean T score based on total cholesterol in total group

However, no correlation was found between this lipid parameter and the bone density indices (T score and BMD) in the total group. No dependencies were found in the separate according to the BMD groups. There was no correlation even after adjusting the analysis to age, duration of MP, WC, BMI and BW in the total group. No correlation was found after exclusion the subjects with statin intake, as well as in an analysis involving only women with TC > 5.18 mmol/l.

When comparing the bone metabolic markers according to TC levels below and above 5.18 mmol/l in the group of osteoporosis, significantly lower levels of Beta Crosslaps (0.44 ± 0.13 ng/ml to 0.59 ± 0.22 ng/ml; p. = 0.029) and DPD/Cr (5.80 ± 1.58 nmol/mmol to 7.88 ± 4.02 nmol/mmol; p = 0.048) were found at levels of TC> 5.18 mmol /l. There was a similar trend to the OC levels, which was not statistically significant (6.27 ± 2.48 ng/ml to 7.12 ± 4.35 ng/ml; p > 0.05). No relationship was found from the correlation analysis between the levels of TC and the bone metabolic markers in the total group, but when excluded the patients taking statin, a weak negative relationship between TC and DPD/Cr was found (r = -0.242; p = 0.042), which remained independent (β = 0.249, p = 0.021) of anthropometric parameters and eGFR. Subsequent multifactorial stepwise analyses did not reveal an independent relationship of this lipid parameter with bone metabolic markers in the total group, among women who did not take statins and among cases with TC > 5.18 mmol/l, even after considering age, duration of MP and anthropometric parameters.

From the comparative analysis of the fracture risks according to the level of TC below and above 5.18 mmol/l a trend to higher 10-year risk of MOF (10.22 ± 5.17 to 8.52 ± 3.69%; p = 0.120) and HF (1.98 ± 2.22 to 1.16 ± 1.18%; p = 0.068) was established at TC > 5.18 mmol/l. The same trend persisted after

excluding statin users. However, no correlations were found between the TC levels and the fracture risks, even after exclusion from the analysis statin users. No dependencies were found in the analyzed according to the BMD groups. Subsequent multifactorial stepwise analyses did not determine the existence of a relationship between TC and fracture risks, even after considering age, duration of the MP and anthropometric parameters in the total group. No dependence was found after exclusion from the analysis statin users. On the other hand, when included in the analyses only the subjects with TC levels above 5.18 mmol/l, a negative association was found with the 10-year risk of HF ($\beta = -0.261$, $p = 0.028$).

Bone and triglycerides (TG). When comparing triglyceride (TG) levels below and above 1.7 mmol/l, no significant difference was found between the analyzed according to BMD groups (Fig. 27). However, fewer cases with lipid levels above 1.7 mmol/l have been reported in women with osteoporosis.

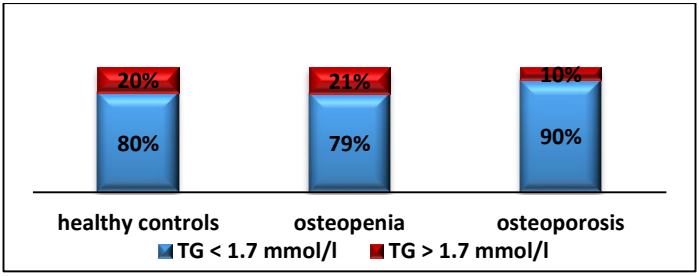

Figure 27. Distribution of triglycerides between the analyzed according to BMD groups

The comparative analysis between the parameters of bone density according to TG levels below and above 1.7 mmol/l in the total group and among statin-free cases revealed significantly higher lumbar spine BMD at TG levels > 1.7 mmol/l (Fig. 28), but this difference was most pronounced in the group of osteopenia (T score L1-L4 -1.14 ± 0.62 to -0.14 ± 0.91, $p = 0.001$).

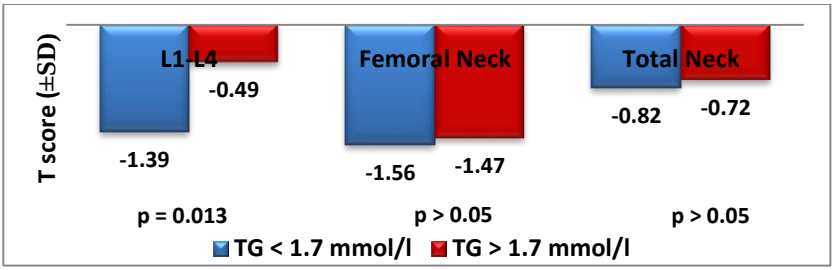

Figure 28. Mean T score based on triglycerides in the total group

On the other hand, the correlation analysis between the TG levels and the bone density indices in the total group revealed a positive dependence with BMD at the level of the lumbar spine (for BMD L1-L4 r = 0.310, p = 0.004), which was maintained after excluding patients taking statins. When comparing the correlation of BMD with TG levels between the analyzed according to BMD groups, it was found that this positive relationship remained significant only in the group of osteopenia (BMD L1-L4 r = 0.571, p <0.001). The loss of positive dependence in the osteoporosis group probably was due to the observed lowest mean TG levels, but in addition, the patients in this group were older, with a longer duration of MP and lower BW. In the group of healthy controls, there was also a loss of the positive correlation, despite the highest mean TG levels. Probably the age, the duration of MP and BW modulate the relationship between TG and BMD parameters at the level of lumbar spine in healthy controls. To determine the independent relationship between TG and BMD at the lumbar spine, we performed a multifactorial stepwise analysis. After considering age, duration of MP, anthropometric parameters and other lipid parameters, it was found that TG remained positively associated with BMD at the level of lumbar spine ($\beta = 0.204$; $p = 0.039$), despite the positive relationship with BW ($\beta = 0.340$; $p = 0.001$) and the negative relationship with the duration of MP ($\beta = -0.260$; $p = 0.008$). On the other hand, after exclusion from the analysis the women taking statins, the dependence was lost.

From the comparative analysis of the bone metabolic markers according to the TG levels below and above 1.7 mmol/l no significant differences were found it total group. In addition, no correlation was found between TG levels and bone metabolic markers in the total group, even after excluding statin users. However, in the osteopenia group, a positive correlation was found between

TG and DPD/Cr levels (r = 0.414, p = 0.009), which remained significant after eGFR adjustment (r = 0.347, p = 0.021), but not after adjustment to the anthropometric parameters (r = 0.263, p = 0.121). Subsequent multifactorial stepwise analyses did not reveal an independent association of the lipid parameter with bone metabolic markers in the total group, as well as among women who did not take a statin, even after considering age, duration of MP and anthropometric parameters.

In total group no significant difference was found from the comparative analysis of the fracture risks according to TG levels below and above 1.7 mmol/l (for MOF 9.60 ± 4.83% to 9.82 ± 4.54%, p> 0.05; for HF 1.66 ± 2.03% to 1.85 ± 1.62%, p> 0.05), but there was a trend to higher fracture risks at TG levels above 1.7 mmol/l. However, after excluding statin users, the trend reversed (for MOF 9.74 ± 5.02% to 8.96 ± 3. 97%, p> 0.05; for HF 1.75 ± 2.12% to 1.65 ± 1.59%, p> 0.05). No correlations were found between the TG levels and the fracture risks, even after excluding statin users. No dependencies were found in the analyzed according to the BMD groups. Subsequent multifactorial stepwise analyses also did not determine a relationship between TG and fracture risks, even after excluding statin users.

Bone and LDL - cholesterol (LDL-C). When comparing LDL-C levels below and above 2.6 mmol/l, no significant difference was found between the analyzed according to BMD groups. However, it was found that in women with osteoporosis the proportion of subjects with lipid levels above 2.6 mmol/l was higher than in healthy controls and cases of osteopenia (Fig. 29).

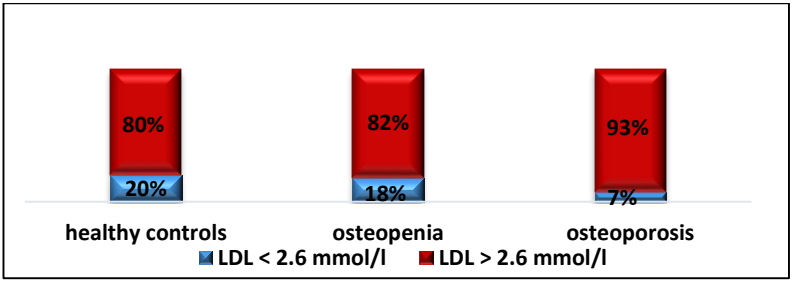

Figure 29. Distribution of LDL-C between the analyzed according to BMD groups

When comparing BMD according to LDL-C levels below and above 2.6 mmol/l, a trend to lower bone density was found at LDL-C levels above 2.6 mmol/l in the total group, which, however, did not reach statistical significance (Fig. 30). The results were similar after considering the statin intake.

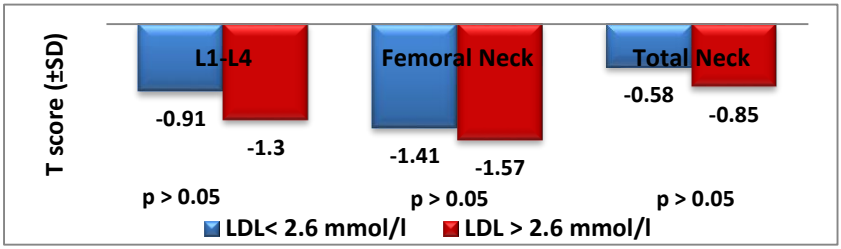

Figure 30. Mean values of T score according to LDL-C levels

In the total group no associations were found in the correlation analysis between LDL-C and the bone density indices in the considered skeletal regions. There was no correlation even after exclusion from the analysis of the women taking statins, as well as in the analysis of the subjects with LDL-C > 2.6 mmol/l. No significant relationships were also found in the analyzed according to the BMD groups.

From the comparative analysis of the bone metabolic markers according to the levels of LDL-C below and above 2.6 mmol/l in the total group no significant differences were found, but there was a trend to higher levels of OC (5.97 ± 2.46 ng/ml to 4.99 ± 2.58 ng/ml, $p > 0.05$) and Beta Crosslaps (0.48 ± 0.17 ng/ml to 0.41 ± 0.11 ng/ml, $p > 0.05$) at LDL-C levels above 2.6 mmol/l. The same trend was observed after taking into account statin intake, as well as in the analyzed according to the BMD groups. However, no correlation was found between LDL-C and the bone metabolic markers in the total group, in cases of LDL-C above 2.6 mmol/l and in subjects not on statin therapy. No correlations were found in the analyzed according to the BMD groups. Subsequent multifactorial stepwise analyses revealed a negative association between LDL-C above 2.6 mmol/l and DPD/Cr ($\beta = -0.222$, $p = 0.037$), regardless of age, duration of MP, anthropometric parameters and eGFR. However, in the total group and among those who did not receive statin therapy, such dependence was missing.

No significant difference was found in the comparative analysis of the fracture risks according to LDL-C levels below and above 2.6 mmol/l in the total group, but there were trends to higher risks at LDL-C above 2.6 mmol/l (for MOF 9.70 ± 4.99% to 9.28 ± 3.36%, $p > 0.05$; for HF 1.78 ± 2.07% to 1.22 ± 1.08%, $p > 0.05$). This trend was mainly observed in the subjects with osteoporosis. The results were similar after excluding statin users. No correlations were found between the LDL-C levels and the fracture risks in the total group, in the group with LDL-C levels above 2.6 mmol/l and among those who did not receive statin therapy. No correlations were found in the analyzed according to the BMD groups. Subsequent multifactorial stepwise analyses also did not determine a relationship between LDL-C and fracture risks even in the group with LDL-C levels above 2.6 mmol/l, regardless of age, duration of MP, anthropometric parameters and statin intake.

Bone and HDL - cholesterol (HDL-C). When comparing HDL-C levels below and above 1.3 mmol/l, it was found that in women with osteoporosis the proportion of persons with lipid levels above 1.3 mmol/l was higher than in healthy controls and cases of osteopenia, but subjects with HDL-C levels above 1.3 mmol/l in the total group were 86% (Fig. 31).

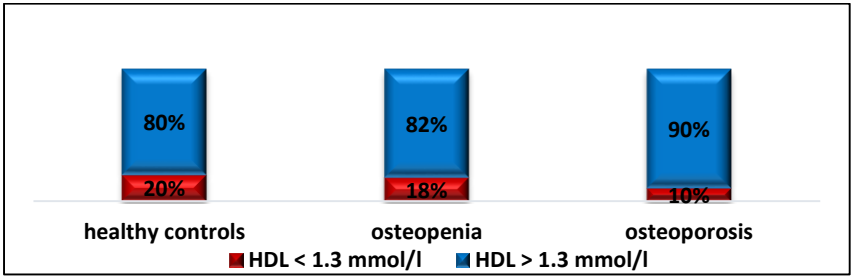

Figure 31. Distribution of HDL-C between the analyzed according to BMD groups

Comparison of BMD according to HDL-C levels below and above 1.3 mmol/l in the total group revealed a trend to lower bone density at the level of lumbar spine when HDL-C levels were above 1.3 mmol/l, which, however, did not reach statistical significance (Fig. 32). The results were similar after excluding from the analysis statin users.

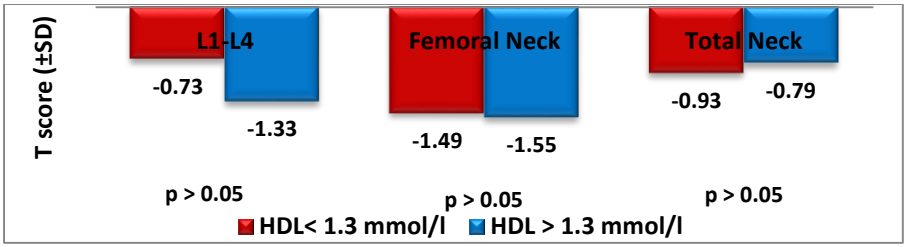

Figure 32. Mean values of T score according to HDL-C levels

Nevertheless, a negative correlation was found between HDL-C levels and BMD at the level of the lumbar spine in the total group (for BMD L1-L4 r = -0.220; p = 0.044). When comparing the correlation dependences of HDL-C with BMD between the analyzed according to BMD groups, it was found that the negative relationship with bone indices at the level of lumbar spine remained significant only in the group of osteopenia (for BMD L1-L4 r = -0.405, p = 0.01). The negative relationship in the total group between HDL-C and BMD at the lumbar spine level was lost when the subjects with HDL-C below 1.3 mmol/l were excluded from the analysis. On the other hand, loss of dependence was observed also after exclusion of statin users. To determine the independent relationship between HDL-C and BMD at the lumbar spine level, we performed a multifactorial stepwise analysis. After considering age, duration of MP and anthropometric parameters, it was found that there was no significant relationship between HDL-C and BMD at the level of lumbar spine despite of negative relationship between BMD and the duration of MP (β = -0.254; p = 0.011), and positive dependence of BMD with BW (β = 0.340; p = 0.001) and TG level (β = 0.204; p = 0.039). Similar results were found after exclusion from the analysis statin users, as well as in the analysis of the cases only with HDL-C > 1.3 mmol/l.

From the comparative analysis of the bone metabolic markers according to the levels of HDL-C below and above 1.3 mmol/l in the total group a lower level of DPD/Cr was found in HDL-C above 1.3 mmol/l (5.77 ± 2.1 nmol/mmol to 7.06 ± 1.92 nmol/mmol, p = 0.05) as the most obvious difference was observed in the group of osteopenia (5.32 ± 1.71 nmol/mmol to 7.60 ± 2.20 nmol/mmol, p = 0.05). The same trend, but without statistical significance, was observed in the total group after excluding statin users. From the correlation analyzes

between HDL-C and the bone metabolic markers in the total group, a negative dependence with DPD/Cr was found ($r = -0.229$; $p = 0.036$). When comparing the correlations between the analyzed according to BMD groups, it was found that the negative relationship remained significant only in the group of osteopenia ($r = -0.387$; $p = 0.015$). On the other hand, when excluding statin users, the negative relationship between HDL-C and DPD/Cr lost its significance. When correlate only the cases with HDL-C > 1.3mmol/l with the bone metabolic markers a negative dependence was found with the level of OC ($r = -0.239$; $p = 0.043$). Subsequent multifactorial stepwise analysis revealed that HDL-C remained negatively associated with DPD/Cr ($\beta = -0.202$; $p = 0.046$), regardless of age, duration of MP, anthropometric parameters, eGFR and other lipid parameters. This relationship lost its significance in the analysis of the cases with HDL-C levels > 1.3 mmol/l and after exclusion statin users from the total group. A negative association was also established between OC and HDL-C ($\beta = -0.237$; $p = 0.031$), regardless of age, duration of MP, anthropometric parameters and other lipid parameters. This relationship was more significant in the analysis of the cases with HDL-C levels > 1.3 mmol/l ($\beta = -0.304$; $p = 0.009$), but lost its significance when excluding from the analysis statin users.

The comparative analysis of the fracture risks according to HDL-C levels below and above 1.3 mmol/l did not find significant differences in the total group, but there was a trend to higher risks at HDL-C above 1.3 mmol/l (for MOF 9.70 ± 4.86% to 9.23 ± 4.23%, $p > 0.05$; for HF 1.72 ± 2.04% to 1.53 ± 1.41%, $p > 0.05$) as the most obvious trend was observed in the groups of osteoporosis and healthy controls. The correlation analyses did not reveal any relationship between HDL-C levels and the fracture risks in the total group, even after considering statin therapy. There was also no association with the fracture risks when analyzing only the subjects with HDL-C level > 1.3mmol/l. No correlations were found in the analyzed according to the BMD groups. However, subsequent multifactorial stepwise analysis revealed that HDL-C was negatively associated with the 10-year risk of HF ($\beta = -0.193$; $p = 0.049$), regardless of age, duration of MP, anthropometric parameters, and other lipid parameters. This dependence was maintained when included in the analysis only subjects with HDL-C levels above 1.3 mmol/l ($\beta = -0.228$; $p = 0.033$), but lost its significance after exclusion from the analysis statin users.

Discussion: The literature data on the relationship between lipid parameters and bone health are contradictory, and our results did not highlight a certain lipid parameter as positive or negative related to bone integrity. Cholesterol is thought to play a dual role in osteoblast differentiation. Accordingly, Li et al. (2019) found that its physiological endogenous levels were essential for osteogenic differentiation of stem cells in bone marrow, but exogenous cholesterol inhibited the differentiation of osteoblasts. These observations suggest that the effect of cholesterol on osteogenesis is more complex and cannot be defined as "good" or "bad". The conducted analyses in the present study between the TC levels and BMD did not suggest significant relationships. Consistent with our results are data from studies by Go J et al. (2012) and Li et al. (2015). On the other hand, Brownbill et al. (2006) found that higher cholesterol levels were positively associated with BMD, and Ersoy et al. (2017) found lower levels of TC in postmenopausal women with osteoporosis compared to a control group of women without osteoporosis. However, Janković D et al. (2010) found reduced BMD in postmenopausal women with elevated TC, and the results of a multivariate regression analysis in the study by Bijelic et al. (2016) revealed that elevated TC levels were a significant independent risk factor for osteoporosis in postmenopausal women. The available data on the relationship between bone metabolism and TC levels in the literature are also inconclusive. A number of authors have found no dependencies (Brownbill 2006, Yamauchi 2015). However, others have found both positive (Majima 2008) and negative (Chen 2013) relationships. The significantly lower levels of bone resorption markers found at TC levels > 5.18 mmol/l in combination with the negative association between the 10-year risk of HF and TC > 5.18 mmol/l found by the multifactorial stepwise analysis in our study could determine favorable relationship between bone integrity and higher levels of TC. Accordingly, Sivas et al. (2009) found that increasing TC by 1 mg/dl reduced the risk of vertebral fracture by 2.2%. On the other hand, a meta-analysis by Ghorabi et al. (2019) found that TC levels were positively related with fracture risk, as increasing it by 50 mg/dl was associated with a 15% higher chance of fracture. However, the trends to lower BMD, lower bone formation and higher fracture risk at TC levels > 5.18 mmol/l that we found did not exclude potential negative dependencies in more severe hypercholesterolemia.

The results of the analyses performed in the present study between TG and BMD indicated that the higher TG levels were independently associated with higher BMD at the level of the lumbar spine, which was most obvious in the group of osteopenia. However, we did not find a clear relationship between TG levels and the markers of bone turnover or the fracture risks, which raised the question of the relationship between the quality of accumulated bone mass at higher TG levels. Therefore, we could not allow the protective effect of hypertriglyceridemia on bone integrity. A number of authors, in accordance with our data, have found a positive correlation between BMD and TG levels in postmenopausal age, but did not report significant relationships with bone microarchitecture or bone metabolic markers (Brownbill 2006, Panahi 2019). On the other hand, Li et al. (2015) did not find a significant correlation between TG and BMD in postmenopausal age. However, Yazdanpanah et al. (2018) found an inverse relationship between TG and BMD at the pelvic level and accepted hypertriglyceridemia as a real factor in the development of osteoporosis. In addition, after analysis of the lipid profile in postmenopausal women, Yamaguchi et al. (2002) found a positive relationship between TG values with previous vertebral fractures. However, in a meta-analysis by Ghorabi et al. (2019), according to our results, no significant relationship has been established between TG levels and the risk of bone fractures.

Data from analyses performed in the present study between LDL-C levels and bone integrity did not suggest a significant association between this lipid parameter and postmenopausal osteoporosis. In accordance with our results, Li et al. (2015) found no correlation between postmenopausal osteoporosis and LDL-C levels. In addition, in the study by Bijelic et al. (2016) LDL-C was not identified as independent risk factors for osteoporosis. On the other hand, Ersoy et al. (2017) found lower levels of LDL-C in postmenopausal women with osteoporosis compared to the control group of women without osteoporosis, and other authors found a negative correlation between LDL-C and postmenopausal osteoporosis (Cui 2012, Wang 2014). However, the trends in our study for lower BMD, higher bone turnover and higher fracture risks at higher LDL-C levels did not exclude potential negative dependences on bone integrity in more pronounced deviations of this lipid parameter.

The initially negative correlation that we found between the HDL-C levels and BMD at the level of lumbar spine remained dependent on the anthropometric

parameters and the duration of MP. However, other authors found a negative relationship with BMD at the femoral region. Li et al. (2015), for example, found significantly lower BMD at the proximal femur and femoral neck when HDL-C levels were higher among postmenopausal women, but in contrast with us they found that this relationship remained independent of age, duration of MP, and BMI. On the other hand, Yamaguchi et al. (2002) found a positive association between HDL-C and BMD at the forearm and spine level regardless of age, duration of MP and BMI, but Wang et al. (2014) found no significant correlation between HDL-C and postmenopausal osteoporosis. However, after multifactorial stepwise analyses in our study, we found that there was no independent relationship between BMD and HDL-C levels in the total group, but we found that higher HDL-C levels were associated with a lower 10-year risk of HF, regardless of the established lower level of bone metabolism. The analysis of the women who did not receive statin therapy also found no association with BMD, but there were no dependencies with either the bone metabolic markers or the fracture risks. On the other hand, in the analysis of the subjects with HDL-C levels > 1.3 mmol/l, a lower level of bone formation was found. However, the relationship with the 10-year risk of HF remained negative. For this reason, we assumed that HDL-C could correlate with better parameters of bone microarchitecture, which would determine a lower fracture risk. The reported divergent associations between HDL-C and bone integrity could be explained by the potential of HDL-C to extract cholesterol from osteoclasts, which reduces their formation and induces apoptosis (Huang X 2018). On the other hand, HDL-C also removes oxysterols from peripheral tissues, which might adversely affect osteogenic differentiation (Yang Y 2018), as oxysterols are involved in the differentiation of MSCs by inhibiting the formation and differentiation of fat cells in bone marrow.

Because the duration of statin use was not assessed in the present study and due to the small number of women in the total group who received antilipemic therapy, we could not comment on the relationship between postmenopausal bone health and HMG-CoAR inhibitors. However, some clinical studies and meta-analyses have found a link between statin use and the improvement of BMD (Liu 2013), as well as reducing the risk of fractures (Helin-Salmivaara 2012). However, other studies and subsequent analyses did not find such a

relationship (LaCroix 2003, Wang 2016). Nevertheless, meta-analysis by Wang et al. (2016) provided evidence that statins were an effective strategy for bone health. Its results suggest that the use of these lipid-lowering drugs might contribute to a significant increase in BMD at the population level. These observations suggest that statins might be clinically relevant in the prevention and treatment of osteoporosis.

4.6 Evaluation and analysis of the relationship between inflammatory markers and bone health

Bone and TNF alpha. The results of the comparative analysis of TNF alpha between analyzed according to the BMD groups revealed that there was a significant difference in the mean values of the proinflammatory cytokine, which increased significantly in the subjects with compromised bone density ($p = 0.015$) (Table 1). In addition, 24% of the women were found to be with TNF alpha levels above the upper reference range of 8 pg/ml, with a higher percentage in the osteoporosis group (Fig. 33).

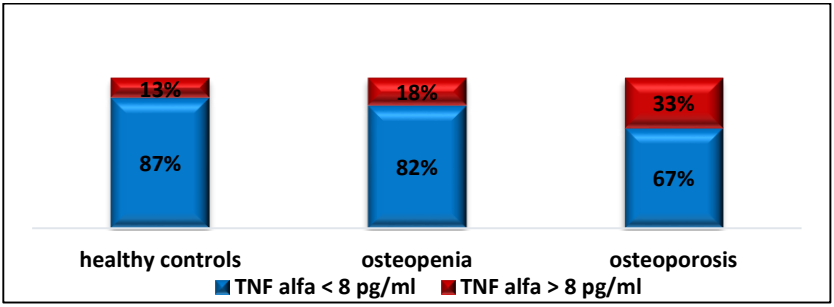

Figure 33. Distribution of TNF alpha above threshold values in the studied according to BMD groups

The comparative analysis of bone density indices with respect to TNF alpha levels below and above 8 pg/ml indicated significantly lower BMD at the lumbar spine at proinflammatory cytokine above thresholds (for BMD L1-L4 0.93 ± 0.15 g/cm^2 to 1.03 ± 0.14 g/cm^2, $p = 0.02$). Subsequent correlation analyses revealed a negative relationship between TNF alpha and BMD at the lumbar spine (for BMD L1-L4 $r = -0.267$; $p = 0.024$). This dependence remained significant after WC and BMI adjustment (for BMD L1-L4 r = -

0.226; p = 0.05), but became insignificant after BW adjustment, which was positively associated with BMD (β = 0.423, p <0.001).

Significantly lower DPD/Cr at inflammatory cytokine levels above threshold value (4.96 ± 1.97 nmol/mmol to 6.28 ± 2.13 nmol/mmol, p = 0.027) were found from a comparative analysis of bone turnover markers according to TNF alpha levels below and above 8 pg/ml. When comparing the levels of TNF alpha according to the determined threshold values of the bone metabolic markers, no significant differences were found, but there was a trend to higher levels of TNF alpha at Beta Crosslaps above 0.44 ng/ml (6.00 ± 9.99 pg/ml to 5.02 ± 5.84 pg/ml, p > 0.05), as well as to lower levels of TNF alpha at DPD/Cr above 5.67 nmol/mmol (4.43 ± 7.23 pg/ml to 6.83 ± 9.42 pg/ml, p > 0.05). No correlation was found between TNF alpha and OC or Beta Crosslaps levels in the total group. A negative relationship (r = -0.267, p = 0.023) was found with DPD/Cr, but this association was observed in the negative relationship between TNF alpha and eGFR (r = -0.349, p = 0.003). In the subsequent correlation analysis after eGFR adjustment, the negative association between TNF alpha and DPD/Cr lost its significance. Multifactorial stepwise analyses of bone turnover markers with TNF alpha and anthropometric parameters revealed no additional correlations.

A comparative analysis of the 10-year risk of MOF and HF according to TNF alpha levels below and above 8 pg/ml revealed trends to higher fracture risks at proinflammatory cytokine levels above the threshold values (for MOF 11.56 ± 5.68% to 9.86 ± 4.48%, p > 0.05, for HF 2.52 ± 3.22% to 1.71 ± 1.49%, p > 0.05), but the differences did not reach statistical significance. However, a subsequent correlation analysis between TNF alpha and fracture risks revealed a significant positive relationship with the 10-year risk of MOF (r = 0.213, p = 0.036) and HF (r = 0.243, p = 0.02). A comparison of the found positive correlations between the fracture risks and TNF alpha according to anthropometric parameters revealed a loss of dependence because of a negative association of BW with the 10-year risk of MOF (β = -0.759, p = 0.001) and HF (β = - 0.813, p <0.001).

TNF alpha and the analyzed clinical and laboratory parameters. Additional relationships between TNF alpha and the analyzed clinical and laboratory parameters were sought. Significantly lower WC (85.41 ± 9.23 cm

to 91.69 ± 10.92 cm, p = 0.035) and lower BW (63.71 ± 10.61 kg to 69.44 ± 10.33 kg, p = 0.05) were found at TNF alpha levels > 8 pg/ml. Subsequent correlations between anthropometric parameters and TNF alpha levels revealed a negative insignificant dependence with BMI (r = -0.200; p = 0.09), while the analysis of the relationship between WC and TNF alpha revealed a negative weak to moderate significant dependence (r = -0.279; p = 0.018). A similar relationship was found between TNF alpha and BW (r = -0.287; p = 0.015). Subsequent multifactorial analysis revealed that BW was the anthropometric parameter that negatively associated with TNF alpha (β = -0.287, p = 0.015), and the correlation with WC lost its significance after BW adjustment.

With respect to 25(OH)D, it was found that vitamin D levels were significantly higher at TNF alpha values > 8 pg/ml (25.54 ± 8.1 ng/ml to 20.80 ± 9.02 ng/ml, p = 0.05). In addition, there was a significant difference in the mean levels of TNF alpha according to the levels of 25(OH)D (Fig. 34).

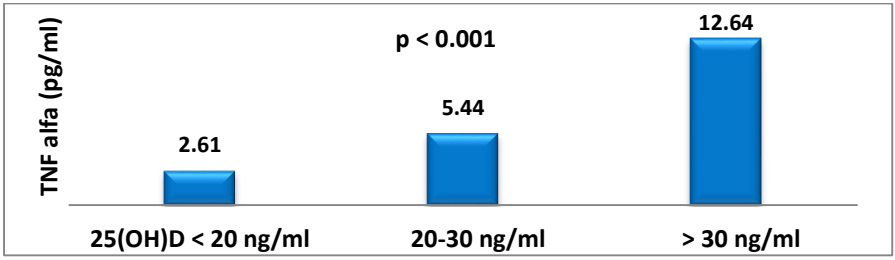

Figure 34. Mean TNF alpha levels according to 25(OH)D levels

Moreover, a moderate positive relationship was found between the proinflammatory cytokine and 25(OH)D levels (r = 0.411; p <0.001), which remained significant after adjustment to the anthropometric parameters (r = 0.383, p = 0.001).

Bone and CRP. The analysis of CRP did not reveal a significant difference between the analyzed according to BMD groups (Table 1). No additional correlations of CRP with BMD were found in the considered skeletal regions. According to the threshold values of the bone metabolic markers, no differences in CRP levels were found. No correlations were found between the inflammatory marker and the bone metabolic markers and the fracture risks.

According to TNF alpha levels significantly higher CRP values (6.90 ± 10.69 mg/l to 3.23 ± 4.24 mg/l, p = 0.041) were found at proinflammatory cytokine levels above 8 pg/ml. However, no correlation was found between the two inflammatory markers.

Discussion: According to the established negative associations of TNF alpha with the parameters of BMD and the positive associations of the proinflammatory cytokine with the fracture risks, we assumed the presence of a negative relationship between TNF alpha and bone integrity. However, no correlations or dependencies were found between CRP and bone integrity.

Although it has been commented on the stimulatory effects of TNF alpha and CRP on osteoclast activity and bone loss (Pfeilschifter 2002, Khosla 2001), we have not found significant independent associations with the markers of bone resorption. In addition, we did not find a positive relationship between WC and BMI with the studied inflammatory markers, despite the known positive dependence between obesity and the levels of circulating inflammatory cytokines (Cao 2011, Guri 2011). We assumed that the reason for these discrepancies in our results was due to the lower percentage (19%) of obese women in the total group.

The established positive relationship between 25(OH)D and TNF alpha does not correspond to the predominant part of the literature data for negative correlation between the two parameters. Our data could be due to skewed results because of the predominance of patients with vitamin D deficiency/insufficiency or the influence of an additional factor not analyzed in the present study. On the other hand, Azizieh F et al. (2016) did not observe a significant correlation between vitamin D levels and serum TNF alpha concentrations. The authors explained this result with vitamin D deficiency and high CRP levels in the analyzed subjects.

4.7 Evaluation and analysis of the relationship between blood pressure and antihypertensive therapy and bone health

Arterial hypertension was defined as systolic BP ≥130 mmHg and/or diastolic BP ≥ 85 mmHg or a history of antihypertensive therapy. In the present study, these values were accepted in view of the criteria for MetS and taking into account the categories of AH defined in 2017 ACC/ANA recommendations.

However, we took into account that BP has not been monitored to confirm the presence of AH in individuals with newly diagnosed AH (nAH). On the other hand, some of the women with nAH fell into the category of high normal BP according to 2018 ESC/ESH recommendations. In this regard, in the subsequent analyses we performed additional calculations by separating the group with nAH from patients with known and treated AH.

In the total group ⅓ of the women were with normal values of BP. Subjects with known AH, who received antihypertensive therapy were 61% of the total group (13% I stage AH, 36% II stage AH and 12% III stage AH). They were with optimal BP levels. Women with nAH were 8% of the total group and were classified as I stage AH.

It is noteworthy that people with nAH were with significantly higher values of SBP and DBP compared to cases without AH and those with known and treated AH (Table 6).

Table 6. Mean values of systolic and diastolic BP (mean ± SD)

	Without AH	**nAH**	**Known AH**	**P value**
Sistolic BP	118±7mmHg	136±8mmHg	126±12mmHg	<0.001
Diastolic BP	74±7mmHg	89±6mmHg	77±8mmHg	<0.001

There was a significant difference (p = 0.001) in the age of women according to the presence of AH and its stages. In subjects without AH the lowest mean age (57.76 ± 6.57y) was reported, while in the group of III stage AH the highest mean age (67.6 ± 4.25y) was found. On the other hand, there was a trend to increase the duration of MP according to the presence of AH and its stages. The postmenopausal period was the shortest in women without AH (9.56 ± 6.41y) and the longest in subjects with stage III AH (14.9 ± 5.36y). However, the differences in duration of MP between the groups according to the presence of AH and its stages did not reach statistical significance (p = 0.06). A significant difference in the analyzed anthropometric parameters according to the presence of AH and its stages was found only in terms of BMI (without AH 25.53 ± 2.51 kg/m^2; NAH 24.55 ± 1.19 kg/m^2; I stage AX 27.24 ± 3.37 kg/m^2; II stage AX 27.9 ± 5.23 kg/m^2; stage III AX 29.52 ± 3.53 kg/m^2, p = 0.02).

Bone mineral density and arterial hypertension

Although the total group was dominated by cases of AH, a higher relative proportion of women with AH was reported in the cases with changes in bone density (osteopenia and osteoporosis) (Fig. 35).

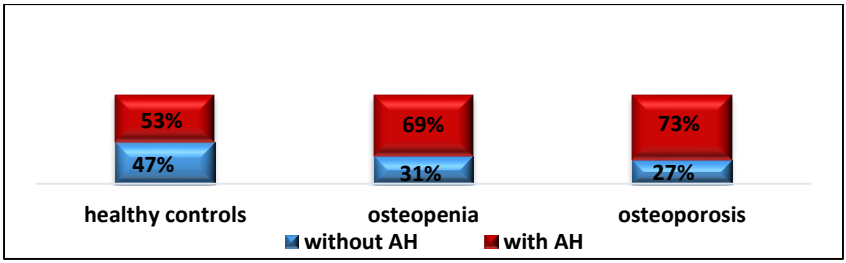

Figure 35. Distribution of arterial hypertension between the analyzed according to BMD groups

In the analysis of the mean values of BMD according to the presence of AH and its stage, several differences were found, although insignificant. The lowest BMD at L1-L4 (BMD 1.00 ± 0.18 g/cm^2), Femoral Neck (BMD 0.78 ± 0.15 g/cm^2) and Total Neck (BMD 0.85 ± 0.15 g/cm^2) was reported in women with stage III AH, which was probably due to the negative impact on the bone of the target organ damage, as well as the older age of patients in this group. The observed better indices in stage II AH at the level of Femoral Neck (BMD 0.85 ± 0.12 g/cm^2) and Total Neck (BMD 0.93 ± 0.13 g/cm^2) compared to the women without AH (BMD Femoral Neck 0.82 ± 0.09 g/cm^2; BMD Total Neck 0.89 ± 0.10 g/cm^2) and compared to the women with stage I AH (BMD Femoral Neck 0.82 ± 0.11 g/cm^2; BMD Total Neck 0.91 ± 0.13 g/cm^2) suggested the presence of a protective effect in the group of stage II AH. In this regard, we hypothesized that the use of some antihypertensive drugs could explain the observed differences, as women with normal BP and those with nAH (included initially in the group of I stage of AH) did not receive antihypertensive therapy.

The potential pleotropic effects of antihypertensive drugs on bone was supported by the established mean values of BMD after separation of women with nAH (respectively untreated). After recalculations in the subjects with treated stage I AH the best values of BMD were reported (BMD L1-L4 1.07 ±

0.12 g/cm^2; BMD Femoral Neck 0.85 ± 0.10 g/cm^2; BMD Total Neck 0.94 ± 0.14 g/cm^2). On the other hand, in persons with nAH the lowest BMD was observed (BMD L1-L4 0.94 ± 0.13 g/cm^2; BMD Femoral Neck 0.76 ± 0.09 g/cm^2; BMD Total Neck 0.85 ± 0.09 g/cm^2). These results were comparable with the established parameters in stage III AH. Additionally, we found a significant difference in the duration of MP between women with nAH and subjects with treated stage I AH (respectively 14.14 ± 6.09y to 8.18 ± 5.56y, p = 0.048) in the absence of a significant difference in age (respectively 60.43 ± 6.65y to 58.27 ± 5.33y, p> 0.05). On the other hand, significant difference in age was found between the subjects with nAH and those with stage III AH (respectively 60.43 ± 6.65y to 67.6 ± 4.25y, p = 0.016) in the absence of significant difference in the duration of MP (respectively 14.14 ± 6.09y to 14.9 ± 5.36y, p> 0.05). These results suggest that untreated AH is associated with worsening BMD parameters, especially in older women and in those with a longer duration of MP.

The conducted correlation analysis revealed a negative association between DBP and BMD at the level of lumbar spine and femoral neck (with BMD L1-L4 r = -0.284, p = 0.009; with BMD Femoral Neck r = -0.232, p = 0.034). These dependences were maintained after considering age, BMI and WC. After considering the duration of MP and BW only the negative correlation between BMD at the level of lumbar spine and DBP remained significant.

Additional analyses revealed that the presence of AH alone was an independent risk factor for bone health in postmenopausal women (OR = 2.14 (0.686-6.703); p = 0.015). Because there was a significant difference in age between subjects with and without AH (respectively 61.8 ± 6.99y to 57.76 ± 6.62 y; p = 0.015), as well as in the analyzed according to BMD groups, we assumed that the negative impact of AH on bone health could be age-modulated. This was confirmed by the calculated higher cumulative risk to bone health after considering the age (OR = 5.66 (0.839-6.462); p = 0.017).

Mean T-scores and BMD were further adjusted to the antihypertensive therapy (Table 7), which included thiazide diuretics (32.1%), ACEi/ARB (46.4%), BB (39.3%) and/or Ca-antagonists (9.5%). Only two women with osteopenia receive a loop diuretic.

Table 7. Mean value of T score/BMD according to the intake of some antihypertensive drugs in the analyzed according to BMD groups (mean; p value)

		Healthy controls			**Osteopenia**			**Osteoporosis**		
		L1-L4	**Femoral Neck**	**Total Neck**	**L1-L4**	**Femoral Neck**	**Total Neck**	**L1-L4**	**Femoral Neck**	**Total Neck**
		T score(±SD)								
ACEi/ ARB	no	0.21	-0.64	0.04	-1.08	-1.67	-0.89	-2.30	-2.01	-1.25
	yes	0.15	-0.38	0.45	-0.72	-1.41	-0.64	-2.42	-2.08	-1.49
	p	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**
Thiazide	no	0.19	-0.54	0.21	-1.02	-1.63	-0.89	-2.18	-1.93	-1.26
	yes	0.16	-0.44	0.36	-0.73	-1.42	-0.55	-2.72	-2.28	-1.60
	p	**>0.05**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**
BB	no	0.02	-0.44	0.31	-1.13	-1.70	-0.96	-2.40	-2.10	-1.47
	yes	0.42	-0.60	0.18	-0.65	-1.36	-0.54	-2.29	-1.94	-1.19
	p	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**
Ca antagonist	no	0.16	-0.58	0.18	-0.97	-1.59	-0.82	-2.35	-2.08	-1.42
	yes	0.18	-0.51	1.40	-0.60	-1.30	-0.47	-2.47	-1.70	-1.37
	p	**> 0.05**	**< 0.05**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**
		BMD (g/cm^2)								
ACEi/ ARB	no	1.21	0.95	1.01	1.05	0.81	0.89	0.88	0.76	0.85
	yes	1.19	0.98	1.06	1.09	0.84	0.93	0.89	0.77	0.82
	p	**> 0.05**	**> 0.05**	**< 0.05**	**< 0.05**	**> 0.05**	**< 0.05**	**> 0.05**	**> 0.05**	**> 0.05**
Thiazide	no	1.20	0.96	1.03	1.06	0.81	0.89	0.90	0.77	0.85
	yes	1.19	0.97	1.05	1.09	0.83	0.93	0.85	0.76	0.81
	p	**> 0.05**	**> 0.05**	**> 0.05**	**> 0.05**	**> 0.05**	**< 0.05**	**< 0.05**	**> 0.05**	**< 0.05**
BB	no	1.18	0.97	1.05	1.04	0.80	0.88	0.87	0.75	0.82
	yes	1.23	0.95	1.03	1.10	0.84	0.94	0.91	0.80	0.86
	p	**< 0.05**	**> 0.05**	**> 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**
Ca antagonist	no	1.19	0.96	1.04	1.06	0.82	0.90	0.88	0.76	0.83
	yes	1.24	1.11	1.19	1.11	0.85	0.95	0.88	0.81	0.89
	p	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**> 0.05**	**< 0.05**	**> 0.05**	**< 0.05**	**< 0.05**

The analysis of the data revealed better BMD parameters at the level of femoral neck and total neck in healthy controls taking ACEi or ARB. In the group of patients with osteopenia, the intake of these drugs was associated with a higher BMD, both in the femoral region and at the level of the lumbar spine. However, no positive effect was found in the group of patients with osteoporosis. Similar results were observed according to thiazides. On the other hand, BBs intake

was positively associated only with lumbar BMD in healthy controls. In patients with osteopenia and osteoporosis, better BMD parameters were observed, both in the femoral region and at the level of lumbar spine. Ca-antagonist intake was positively associated with BMD, both in the femoral region and at the level of lumbar spine in healthy controls and in patients with osteopenia. In patients with osteoporosis, there was a positive association with BMD only at the level of femoral neck and total neck.

Markers of bone metabolism and arterial hypertension

No significant difference was found between the levels of bone metabolic markers according to the presence of AH or its stage. In addition, no correlations were found between the levels of bone metabolic markers and the values of BP in the total group and in the analyzed according to BMD groups. However, significant differences were found in the levels of bone metabolic markers after considering the intake of antihypertensive drugs in women with osteoporosis (Fig. 36).

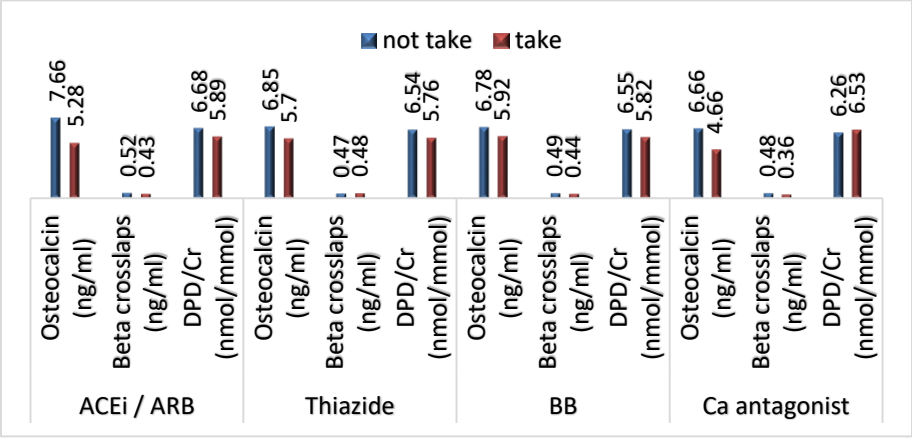

Figure 36. Comparative analysis of the levels of osteocalcin, Beta Crosslaps and DPD/Cr according to medication intake in women with osteoporosis

The results revealed that women with osteoporosis taking the discussed antihypertensive drugs had significantly lower levels of osteocalcin ($p < 0.05$). The same trends were maintained for Beta Crosslaps and intake of ACEi or

ARB, BBs and/or Ca-antagonists, as well as for the ratio of DPD/Cr and intake of ACEi or ARB, thiazide diuretic and/or BBs. However, there was no significant difference ($p > 0.05$) in the levels of Beta Crosslaps according to the intake of thiazide diuretic and in the DPD/Cr ratio according to the intake of a Ca-antagonist.

Fracture risk and arterial hypertension

In assessing the risk of fractures, a significant difference was found according to the presence of AH and its stages (Table 8). The lowest risks were reported in women with stage I AH and in cases without AH, while in stage III AH the fracture risks were the highest and comparable to those in women with nAH.

Table 8. Mean fracture risk values according to AH stage (mean±SD)

Fracture risk	No AH	nAH	Stage of AH			P value
			first	second	third	
10-year risk of MOF (%)	8.45±3.65	12.4±4.11	7.78±3.28	9.34±4.39	13.70±7.30	0.008
10-year risk of HF (%)	1.23±0.92	3.16±3.21	0.71±0.78	1.58±1.50	3.29±3.44	0.003

Subsequent correlation analysis indicated a positive association between DBP and both the 10-year risk of MOF ($r = 0.252$; $p = 0.021$) and HF ($r = 0.282$; $p = 0.009$). This dependence was maintained for the two fracture risks after adjustment to age, BW, BMI and WC. After considering duration of MP, only the correlation between DBP and 10-year risk of HF remained positive. No additional differences and dependencies were found between the risk of fractures and the intake of antihypertensive drugs.

Ca-P metabolism and arterial hypertension. The analysis of the levels of urinary calcium revealed a moderate positive dependence with BP ($r = 0.363$; $p = 0.022$) after excluding thiazide users, i.e. greater urinary calcium loss at higher BP values. On the other hand, the correlation analysis of serum P levels with BP revealed a negative dependence ($r = -0.300$; $p < 0.05$), i.e. elevated BP were associated with lower serum P levels. Similar result was found for phosphaturia, but it was not significant ($r = -0.126$; $p > 0.05$).

Associations between arterial hypertension and inflammatory markers. The analysis of TNF alpha according to the presence of AH indicated that patients with AH had elevated levels of the inflammatory cytokine compared

to those without AH (5.91 ± 9.31 pg/ml to 3.53 ± 4.38 pg/ml, p > 0.05), as women with nAH were with significantly higher levels of TNF alpha compared to those without AH (9.99 ± 9.63 pg/ml to 3.53 ± 4.38 pg/ml, p = 0.017). In addition, TNF alpha levels were found to decrease, albeit insignificantly, with increasing AH stage, as in stage III AH were lower than in the group of subjects without AH (p> 0.05) (Fig. 37). However, no correlation of SBP and DBP with the proinflammatory cytokine has been found.

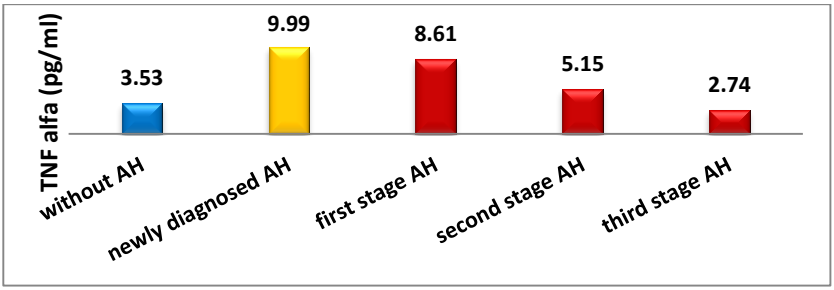

Figure 37. Mean TNF alpha levels according to the presence of AH and its stage

When comparing TNF alpha levels according to the drugs intake, it was found that the use of statins and ACEi/ARB was associated with a significant decrease in proinflammatory cytokine values (p < 0.001) (Fig. 38).

In the analysis of the women according to the stage AH and the intake of antihypertensive drugs and statins, it was found that all patients in stage III AH received ACEi/ARB, and 70% of them also received statins (p <0.001), which could explain the lower TNF alpha levels even below those in the subjects without AH. On the other hand, only 55% of women with stage I AH recieved ACEi/ARB, and only one woman recieved statin. In the group of stage II AH, 77% of women took ACEi/ARB and only 10% took statin.

No differences and dependencies were found between CRP and BP values, the presence of AH or its stage.

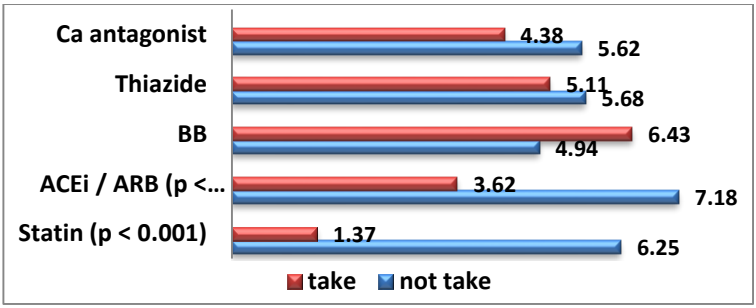

Figure 38. Mean TNF alpha levels (pg/ml) according to drugs intake

Discussion: According to the results obtained in the present study, BP and AH appear to be additional determinants of bone health in postmenopausal age. Our data are comparable to the results of a number of other clinical studies and meta-analyses (Yang 2014, Li 2017, Ye 2017, Chai 2021), but do not overlap with the conclusion of Hanley et al. (2003) that AH is associated with an increase in bone mass, as well as with the conclusion of Hijazi et al. (2020), which did not establish a relationship between osteoporosis and AH in adult postmenopausal women. The negative correlation found between DBP and BMD, with a positive relationship between DBP and fracture risks, corresponds to the results of Jeon et al (2011), while other authors reported a negative impact of SBP on bone health (Cappuccio1999). In addition, we found that not only higher BP, but also the presence of AH and its stage could be relevant to postmenopausal osteoporosis, as in women without AH and in those with stage I AH who were on medical treatment, the highest values of BMD at the level of the lumbar spine and in the area of the femoral region were reported, as well as the lowest risks of fractures. On the other hand, in subjects with nAH (respectively untreated) and in the group of stage III AH, the lowest values of BMD and the highest fracture risks were reported. These results suggest that untreated AH is associated with deteriorating bone integrity, which we observed especially in older women and in those with a longer duration of MP. In addition, our data support the statements about the potential pleotropic effects of antihypertensive drugs on bone (Hijazi 2020, Ghosh 2014). The better BMD indices found in different skeletal regions in the group of healthy controls and among women with osteopenia taking ACEi/ARB, thiazide diuretic, BB and/or Ca-antagonist raise the question of

the importance of these antihypertensive drugs in prevention and prophylaxis of osteoporosis. On the other hand, the lack of such observations in the group of women with osteoporosis taking ACEi/ARB and/or thiazide diuretics suggests a loss of protective effect in osteoporosis or negative impact of additional factors such as older age, longer duration of MP or concomitant comorbidities. In addition, we found that the intake of the antihypertensive drugs in the group of osteoporosis was associated with a lower level of bone turnover.

The commented pathogenetic mechanisms that negatively link higher BP and AH with deteriorating bone health are associated with abnormal cytokine levels, increased RAAS activity at the systemic and local level, abnormalities in Ca-P metabolism, increased sympathetic tone, vascular disorders (Do Carmo 2020). Accordingly, we found higher serum levels of TNF alpha in patients with AH compared to individuals without AH. On the other hand, we found that statin and ACEi/ARB intake were associated with significantly lower levels of the proinflammatory cytokine. In addition, we found that BP correlated positively with urinary calcium loss and negatively with serum P. The established positive dependence of calcium in urine with BP after excluding thiazide users supports one of the possible mechanisms by which AH worsens bone health, drawing attention to the potential benefits of thiazide diuretics to bone (Sigurdsson 2001, Schoofs 2003, Aung 2011). These data are consistent with the found lower urinary calcium in postmenopausal women treated with thiazide by Reid et al. (2000), as well as with the results of Sigurdsson et al (2001), who found a higher BMD in older women taking such drugs. The negative relationship between serum P and BP could be explained by increased sympathetic-adrenal activity in subjects with AH, as catecholamines lead to a net change in P by directing it from the extracellular to the intracellular space (Body 1983). On the other hand, higher bone density was found in BB and Ca-antagonist users, probably due to decreased sympathetic tone and decreased vascular resistance from these drugs. Our results are supported by the data of Pasco et al. (2004), who reported an increase in BMD in women > 50 years of age receiving BB. Literature data on the effect of Ca-antagonists on bone health are limited and unconvincing, but direct effects of this class of drugs at the level of osteoclasts and osteoblasts are also discussed (Gradosova 2012, Ushijima 2010, Kosaka 1998).

4.8 Evaluation and analysis of the relationship between metabolic syndrome and bone health

The metabolic syndrome is defined according to the combined criteria of 2009 IDF, AHA, NHBLI, WHF and IASO. In the general sample without MetS were 51.2% of the cases, and the subjects with MetS were 48.8%. In the group with MetS the cases with 3 components of the syndrome predominate (51.2%), while the women with fully-developed MetS were 12.2%. On the other hand, in the group without MetS 53.5% of the cases had 2 components of the syndrome. The distribution of MetS in the analyzed according to BMD groups did not show a significant difference, as about half of the healthy controls and those with osteopenia and osteoporosis were with MetS. As expected, from the comparative analysis a significant difference in the values of the components of the syndrome between subjects with and without MetS was reported (Table 9). Significant differences were also reported in some additional anthropometric, metabolic and inflammatory parameters between women with and without MetS (Table 10).

Table 9. Comparative analysis of the components of the metabolic syndrome in the groups according to the presence or absence of the syndrome

Parameter		**With MetS**	**Without MetS**	**P value**
WC (cm)	mean±SD	95.36±10.87	86.86±10.39	**< 0.001**
SBP (mmHg)	mean±SD	128.78±11.82	119.42±9.35	**< 0.001**
DBP (mmHg)	mean±SD	79.27±8.33	75.46±6.97	**0.026**
TG (mmol/l)	mean±SD	1.42±0.57	0.94±0.27	**< 0.001**
HDL-C (mmol/l)	mean±SD	1.57±0.40	1.78±0.36	**0.015**
BG 0 min (mmol/l)	mean±SD	5.91±0.59	5.33±0.36	**<0.001**

Table 10. Comparative analysis of anthropometric, metabolic, inflammatory and hormonal parameters according to the presence or absence of MetS

Parameter		**With MetS**	**Without MetS**	**P value**
Age (y)	mean±SD	61.26±6.51	59.83±7.57	0.357
Duration of MP (y)	mean±SD	11.53±6.67	11.12±6.73	0.781
BMI (kg/m^2)	mean±SD	28.64±4.58	25.42±2.76	**< 0.001**
BW (kg)	mean±SD	72.51±12.45	65.91±9.19	**0.007**
smoking	да	34.1%	25.6%	0.268
BG 120 min (mmol/l)	mean±SD	6.46±2.25	5.61±1.11	**0.030**

Insulin 0 min (μIU/ml)	mean±SD	10.16±5.95	7.23±3.77	**0.008**
Insulin 120 min (μIU/ml)	mean±SD	62.28±44.68	43.98±21.62	**0.018**
HOMA index	mean±SD	2.68±1.63	1.73±0.96	**0.002**
TC (mmol/l)	mean±SD	5.72±1.28	5.69±0.89	0.889
LDL-C (mmol/l)	mean±SD	3.5±1.18	3.5±0.79	0.989
25(OH)D (ng/ml)	mean±SD	20.56±7.12	24.09±10.53	0.078
CRP (mg/l)	mean±SD	5.22±6.44	2.71±5.40	**0.05**
TNF alfa (pg/ml)	mean±SD	4.01±5.58	6.99±10.35	0.132
Uric acid (mcmol/l)	mean±SD	293.26±74.81	274.63	0.242
Ca (mmol/l)	mean±SD	2.42±0.10	2.42±0.09	0.885
Ca^{2+} (mmol/l)	mean±SD	1.18±0.08	1.18±0.07	0.799
Ca in urine (mmol/24ч)	mean±SD	3.53±3.03	3.23±2.44	0.609
P (mmol/l)	mean±SD	1.16±0.15	1.18±0.13	0.655
P in urine (mmol/24ч)	mean±SD	20.17±16.68	20.42±13.91	0.943
PTH (pg/ml)	mean±SD	51.42±20.26	50.45±16.78	0.812
TSH (μIU/ml)	mean±SD	1.63±0.78	1.84±1.33	0.377

The results of the comparative analysis of T score and BMD according to the presence and absence of MetS revealed a trend to higher bone density in women with MetS, as a significant difference was found for BMD at Total Neck level (Table 11).

Table 11. Comparative analysis of BMD according to the presence of metabolic syndrome (mean ± SD)

Sceletal region	**T score (±SD)**			**BMD (g/cm^2)**		
	With MetS	**Without MetS**	**P value**	**With MetS**	**Without MetS**	**P value**
L1	-1.32±1.05	-1.69±1.17	0.129	0.97±0.13	0.93±0.14	0.144
L2	-1.46±1.33	-1.82±1.33	0.229	1.02±0.16	0.98±0.16	0.238
L3	-0.82±1.40	-1.27±1.40	0.146	1.10±0.17	1.05±0.17	0.163
L4	-0.83±1.55	-1.38±1.50	0.098	1.10±0.19	1.04±0.18	0.111
L1-L4	-1.06±1.24	-1.41±1.26	0.202	1.05±0.15	1.00±0.15	0.132
Femoral Neck	-1.44±0.86	-1.64±0.75	0.252	0.84±0.12	0.82±0.11	0.494
Upper Neck	-1.33±1.03	-1.49±0.83	0.452	0.66±0.12	0.64±0.10	0.452
Lower Neck	-	-	-	1.01±0.13	0.97±0.12	0.155
Ward‘s triangle	-1.99±0.98	-2.08±0.86	0.542	0.65±0.13	0.64±0.11	0.645
Trochanter	**-0.66**±0.99	**-1.12**±0.93	**0.029**	**0.77**±0.11	**0.72**±0.11	**0.033**
Shaft	-	-	-	1.09±0.23	1.05±0.14	0.378
Total Neck	**-0.60**±0.99	**-1.01**±0.91	**0.05**	**0.93**±0.13	**0.88**±0.11	**0.05**

The analysis of bone density between the analyzed according to BMD groups revealed that women with osteopenia and MetS had significantly higher BMD at the level of lumbar spine compared to individuals in the same group, but without MetS (BMD L1-L4 1.11 ± 0.1g/cm^2 to 1.02 ± 0.07g/cm^2, p = 0.006).

According to the levels of bone metabolic markers, no significant differences were found between individuals with and without MetS in the total group. No consistent dependences were found according to the number of MetS components, but some differences were found in the analyzed according to the BMD groups. In women with osteoporosis and MetS, we reported significantly lower values of OC ($p < 0.01$) and Beta Crosslaps ($p < 0.05$) compared to individuals in the same group, but without MetS. The analysis of DPD/Cr revealed a significantly higher level in people with osteopenia and MetS ($p < 0.01$) compared to women in the same group, but without MetS. In the osteoporosis group the difference was insignificant, which we associate with lower glomerular filtration bacause of lower BMI and older age of individuals in this group.

No significant difference was found in the fracture risks according to the presence of MetS in the total group and in the analyzed according to the BMD groups. No consistent relationships were found with respect to the number of MetS components.

From the comparative analysis of the correlations between the components of MetS and the analyzed bone parameters in persons with and without MetS in the total group some differences were reported. In women with MetS, higher WC was associated with higher BMD in the lumbar spine ($r = 0.282$; $p = 0.037$) and in the femoral region ($r = 0.307$; $p = 0.025$), despite a positive correlation with the urinary marker of bone resorption DPD/Cr ($r = 0.363$; $p = 0.01$). Higher TG levels were also associated with higher BMD, but only in the lumbar spine ($r = 0.306$; $p = 0.026$) as well as with higher DPD/Cr levels ($r = 0.291$; $p = 0.032$). On the other hand, DBP correlated negatively with BMD at the level of lumbar spine ($r = -0.384$; $p = 0.007$) and at the femoral neck ($r = -0.308$; $p = 0.025$). Higher DBP was also associated with higher fracture risks (for MOF $r = 0.402$; $p = 0.005$; for HF $r = 0.411$; $p = 0.004$). In addition, HDL-C levels correlated negatively with BMD at the lumbar spine ($r = -0.291$; $p = 0.033$), but higher lipid levels were associated with lower levels of the bone

resorption marker DPD/Cr (r = -0.358; p = 0.011). An additional positive correlation was reported between fasting BG and the 10-year risk of MOF (r = 0.303; p = 0.027). In women without MetS, only higher WC was associated with higher BMD, but solely in the femoral region (r = 0.394; p = 0.004). There was no correlation with the bone turnover markers and fracture risks. On the other hand, higher values of DBP were associated with lower BMD at the level of the lumbar spine (r = -0.295; p = 0.028) and with higher values of the serum marker of bone resorption Beta CrossLaps (r = 0.265; p = 0.043), but there was no correlation with fracture risks. In addition, higher levels of HDL-C (r = -0.295; p = 0.027) and fasting BG (r = -0.356; p = 0.019) were associated with lower levels of the marker of bone formation OC.

Subsequent multifactorial stepwise analysis between MetS components and analyzed bone parameters revealed that in MetS higher DBP values were associated independently from the other components of the syndrome with lower BMD at lumbar spine (for BMD L1-L4 β = -0.384, p = 0.013) and with higher fracture risks (for MOF β = 0.402, p = 0.009, for HF β = 0.411, p = 0.008). On the other hand, the independent positive relationship of WC with DPD/Cr (β = 0.310, p = 0.04) and the independent negative relationship of HDL-C with DPD/Cr (β = -0.304, p = 0.043) lost their significance after considering eGFR. From the multifactorial stepwise analysis between the components of MetS and the analyzed bone parameters in the subjects without MetS it was established that higher values of DBP were also associated with lower BMD at the level of lumbar spine (for BMD L1-L4 β = - 0.343, p = 0.024), and higher WC was associated with higher BMD in the femoral region (for BMD Total Neck β = 0.394, p = 0.009; for BMD Femoral Neck β = 0.404, p = 0.007). On the other hand, higher fasting BG was associated with a lower level of the marker of bone formation OC (β = -0.356, p = 0.019).

Because of the established additional significant differences in BMI, BW, BG at 120 min, fasting insulin and insulin at 120 min, HOMA index and CRP between individuals with and without MetS, a subsequent comparative analysis of correlations between these clinical and laboratory parameters and analyzed bone parameters were performed in persons with and without MetS. The analyses revealed that in women with MetS, higher BW was associated with higher BMD in the lumbar spine (for BMD L1-L4 r = 0.403; p = 0.005) and in the femoral region (for BMD Femoral Neck r = 0.259, p = 0.005; for

BMD Total Neck r = 0.327, p = 0.019), as well as with lower fracture risks (for MOF r = -0.367, p = 0.009; for HF r = -0.357, p = 0.011), regardless of the negative correlation with the marker of bone formation OC (r = -0.279; p = 0.039) and the positive correlation with the urinary marker of bone resorption DPD/Cr (r = 0.262; p = 0.049). In addition, higher BMI was associated with higher BMD at the level of lumbar spine (r = 0.341; p = 0.015) as well as with lower fracture risks (for MOF r = -0.276; p = 0.04; for HF r = -0.317; p = 0.022) and a negative correlation was found with the serum marker of bone resorption Beta Crosslaps (r = -0.264; p = 0.048). On the other hand, higher insulin levels at 120 min were associated with lower BMD at the femoral level (for BMD Femoral Neck r = -0.286, p = 0.035) and with higher fracture risks (for MOF r = 0.326), p = 0.019; for HF r = 0.298, p = 0.029). An additional positive correlation was reported between BG at 120 min and the 10-year risk of MOF (r = 0.293, p = 0.032). In women without MetS, higher BW was also associated with higher BMD in the lumbar spine (for BMD L1-L4 r = 0.459, p = 0.001) and in the femoral region (for BMD Femoral Neck r = 0.622, p < 0.001, for BMD Total Neck r = 0.569, p <0.001), as well as with a lower 10-year risk of HF (r = -0.422; p = 0.002), despite the lack of correlation with bone turnover markers. Higher BMI was associated with higher BMD at the level of Femoral Neck (for BMD r = 0.399; p = 0.004) and Total Neck (for BMD r = 0.403; p = 0.004), despite the positive correlation with the bone resorption marker DPD/Cr (r = 0.275; p = 0.037), but with no dependence on fracture risks. In addition, higher fasting insulin and HOMA index in subjects without MetS were associated with higher BMD in lumbar spine region (for insulin r = 0.381, p = 0.006; for HOMA index r = 0.392, p = 0.05) and in the femoral region (for insulin r = 0.425, p = 0.002; for HOMA index r = 0.444, p = 0.001) as well as with lower fracture risks (for MOF and insulin r = -0.426, p = 0.002; for HF and insulin r = -0.382, p = 0.006, for MOF and HOMA index r = -0.394, p = 0.004, for HF and HOMA index r = -0.352, p = 0.01), despite the lack of correlations with bone turnover markers. However, in contrast to women with MetS, higher insulin at 120 min in subjects without MetS were associated with a lower 10-year risk of MOF (r = -0.257, p = 0.048). An additional positive correlation was reported between BG at 120 min and the fracture risks (for MOF r = 0.263, p = 0.044; for HF r = 0.297, p = 0.027).

To determine the independent relationships of bone integrity with the components of MetS and the additionally analyzed clinical and laboratory parameters, we performed a subsequent multifactorial stepwise analysis. The results in the subjects with MetS revealed that higher BW was associated with higher BMD at the level of lumbar spine ($\beta = 0.392$, $p = 0.008$) and proximal femur ($\beta = 0.327$, $p = 0.037$), and higher levels of TG were associated with higher BMD in the lumbar spine ($\beta = 0.292$, $p = 0.045$). However, there were no dependencies with bone turnover markers and fracture risks. In addition, no dependences of BMD, bone turnover markers and fracture risks with BMI and WC have been identified. On the other hand, higher DBP values were associated with higher fracture risks (for MOF $\beta = 0.382$, $p = 0.01$; for HF $\beta = 0.411$, $p = 0.008$), and higher insulin levels at 120 min were associated with a higher 10-year risk of MOF ($\beta = 0.303$, $p = 0.039$). Subsequent calculations revealed that the higher DBP increased the 10-year risk of HF by 16.9%, and the combination of increased DBP and higher insulin at 120 min increased the 10-year risk of MOF by 25.2%. Additional negative dependence of HOMA index with BMD at the level of lumbar spine ($\beta = -0.288$, $p = 0.049$) and positive dependence of the 10-year risk of MOF with fasting BG were reported when considering age and duration of MP ($\beta = 0.341$, $p = 0.005$), regardless of the negative association of BW with fracture risk ($\beta = -0.264$, $p = 0.028$). On the other hand, the duration of MP remained independently associated with lower BMD and higher fracture risks. In subjects without MetS, higher BW was found to be associated with higher BMD in the lumbar spine ($\beta = 0.849$, $p < 0.001$) and in the femoral region ($\beta = 0.471$, $p = 0.001$), as well as with lower 10-year risk of HF ($\beta = -0.882$, $p < 0.001$), despite the lack of dependence with the bone metabolic markers. It is noteworthy that the positive relationship between BMD and BW was stronger in people without MetS than those with MetS. On the other hand, higher WC in the absence of MetS was associated with lower BMD at the lumbar spine ($\beta = -0.533$, $p = 0.008$), and higher BMI was associated with a higher 10-year risk of HF. ($\beta = 0.570$, $p = 0.003$). In addition, higher DBP values were associated with lower lumbar spine BMD ($\beta = -0.351$, $p = 0.008$) and a higher 10-year risk of HF ($\beta = 0.278$, $p = 0.019$), while higher HDL-C levels were associated with a lower 10-year risk of HF ($\beta = -0.308$, $p = 0.011$). However, there was a positive relationship between HOMA index and BMD in the femoral region ($\beta = 0.277$, $p = 0.042$) and a lower 10-year risk of MOF at higher fasting insulin levels ($\beta = -0.426$, $p =$

0.04) as well as a lower 10-year risk of HF at higher levels of insulin at 120 min (β = -0.353, p = 0.006), but no dependence between insulin levels and HOMA index with bone turnover markers was found. On the other hand, the higher fasting BG was associated with a lower level of the bone formation marker OC (β = -0.356, p = 0.019), and the higher BG at 120 min was associated with a higher 10-year risk of HF (β = 0.426, p = 0.001). These results suggest a negative association of bone integrity with higher BG levels in women without diabetes, but draw further attention to the association of OC with glucose homeostasis. Additional negative dependence of the HOMA index with a 10-year risk of MOF (β = - 0.296, p = 0.009) was reported when considering age and duration of MP, regardless of the positive relationship between age and fracture risks. Age was also positively associated with DPD/ Cr (β = 0.321, p = 0.043), independent of eGFR.

Discussion: Our results revealed that postmenopausal women with MetS were with higher bone mass at the proximal femur level than those without MetS. Additionally, we found a higher BMD at the level of lumbar spine in women with osteopenia and MetS compared to subjects in the same group, but without MetS. On the other hand, we found a lower level of bone turnover in women with osteoporosis and MetS compared to subjects in the same group, but without MetS. However, between the whole group with MetS and the whole group without MetS we did not report differences in the bone turnover markers and fracture risks.

Despite the established positive correlations of T score and BMD in different skeletal regions with WC, we do not accept that MetS-related visceral obesity is positively associated with BMD. However, multifactorial analyses revealed that the reported positive relationships between bone mass and WC were determined by higher BW and possibly the associated greater mechanical load on the skeleton. In addition, BW and BMI, but not WC, in the cases with MetS, correlated negatively with fracture risks, despite established negative correlations of BW and BMI with OC and Beta Crosslaps, respectively. The results of the present study further revealed that higher TG levels were also associated with higher BMD at the level of the lumbar spine, regardless of the other components of the syndrome and the concomitant anthropometric and metabolic changes. In addition, lower levels of HDL-C were associated with higher BMD at the level of lumbar spine, but this relationship lost its

significance after considering the other components of the syndrome and the concomitant anthropometric and metabolic changes. However, we did not report significant independent relationships for both lipid parameters with the bone metabolic markers and fracture risks, which raises the question of the relationship between bone microarchitecture and these lipid parameters. On the other hand, higher DBP values were associated independently of the other components of the syndrome with lower BMD in different skeletal regions. However, the dependence between DBP and bone mass lost its significance after considering the concomitant anthropometric and metabolic changes, probably due to the counterbalancing effect of BW and TG. On the other hand, higher insulin levels at 120 min in the women with MetS were associated with lower BMD at the level of the femoral neck, but this dependence was lost after considering the components of the syndrome. However, the multivariate analysis of the relationships between MetS components and concomitant anthropometric and metabolic changes with the analyzed bone parameters in women with MetS revealed that higher DBP was independently associated with higher fracture risks and higher insulin levels at 120min were associated independently with a higher 10-year risk of MOF, but there were no relationships with bone turnover markers for both parameters. Moreover, the negative correlations found between fracture risks and BW were losing their significance, probably due to the counterbalancing effect of DBP and the progression of IR. The established positive correlations of the 10-year risk of MOF with fasting BG and BG at 120 min in women with MetS also lost their significance in the multivariate analysis at the expense of DBP and insulin levels at 120 min. However, after considering age and duration of MP, it was found from the multifactorial analysis that HOMA index was negatively associated with BMD at the level of lumbar spine, and fasting BG was associated with a higher 10-year risk of MOF in MetS, regardless of the positive relationship with the duration of MP and the negative dependence on BW. In accordance with our results, Alissa et al. (2014) found higher BMD at the proximal femur level in the postmenopausal women with MetS compared to those without MetS. The authors noted that WC was the component of MetS that was positively associated with bone density, but like us, they found after a multivariate analysis that BW determined this relationship. Chin et al. (2020) also found a positive relationship between MetS and BMD, but further clarified that this relationship was mediated mainly by BMI and the associated greater

mechanical stress on bone. However, the authors emphasized the negative relationship between WC and BMD and noted, in contrast to us, that higher levels of TG in MetS were positively associated with osteoporosis. However, MetS was not identified as a significant risk factor for osteoporosis in this study. But other studies have shown a negative relationship between MetS and bone health. An analysis by von Muhlen D et al. (2007) found that people with MetS had lower BMD at the femoral region after considering BMI. It was further noted that the incidence of osteoporotic nonvertebral fractures was significantly higher in the analyzed adult women with MetS. The results of Kim et al. (2010), revealed that people with MetS were with a lower BMD at the femoral region than those without MetS, and among all components of MetS as the most significant negative predictor of BMD, the authors distinguish WC. On the other hand, Jeon et al. (2011) found that MetS was negatively associated with BMD at the level of lumbar spine and femoral neck in postmenopausal women. However, the authors identified CRP and DBP as independent factors for lower bone mass. But Abourazzak et al. (2016) noted in their study that MetS was not associated with postmenopausal BMD, but did not rule out a negative relationship between bone mass and the components of MetS according to the degree of their manifestation. In addition, the authors pointed out that abdominal obesity in postmenopausal women is the most important factor associated with bone loss, especially at the level of lumbar spine. In the same study, it was found in accordance with our data that there was an independent positive relationship between TG and BMD in MetS, but at the level of proximal femur. Similar to our results, Rark et al. (2010) found in postmenopausal women with MetS negative relationship between HDL-C and BMD, but not only at the level of lumbar spine. The negative association was established also at the femoral neck level. On the other hand, Muka et al. (2015) found that HDL-C was associated independently with higher BMD in the femoral region in women with MetS, but found no association with fracture risks. However, the question of fracture risk in MetS remains open. We did not find a significant difference between postmenopausal fracture risks according to the presence or absence of MetS, but we identified components of the syndrome and concomitant metabolic changes that were associated with higher fracture risks. In this regard, we assumed that the fracture risk might be increased in MetS according to the number, combination and degree of manifestation of its components. On the other hand, a meta-analysis of Yang

(2016), which included five prospective studies, revealed that MetS was associated with a significantly lower risk of fractures. However, when this analysis was limited to four of the studies, the association lost its significance after considering BMI. Other studies have discussed the association of MetS-related IR, which could increase the risk of fractures through additional effects on bone strength (Yang 2018), regardless of the association with BMD. However, we found in the cases with MetS that the HOMA index was negatively associated with lumbar spine BMD, and the higher insulin levels at 120 min were associated with lower femoral neck BMD and with higher fracture risks, regardless of BW and BMI. We hypothesized that these relationships with insulin levels at 120 min were due to the progression of IR, as assessed by concomitant compensatory hyperinsulinemia during OGTT. In connection with these results, we assumed a loss of osteoanabolic effect of insulin because of a loss of insulin sensitivity at a bone level in IR conditions. It is further commented in the literature that IR could increase the risk of fractures, regardless of BMI, as there were preconditions for increased frequency of falls. For example, there was evidence that IR results in lower muscle mass (Alemán-Mateo 2014, Lee 2015) and strength (Abbatecola 2005, Barzilay 2009), which could lead to greater instability. On the other hand, it is clear that with the development and progression of T2DM, additional negative factors develop, which predispose to a decrease in bone mass and deterioration of bone microarchitecture. However, we found that even before the onset of T2DM fasting BG and BG at 120 min were negatively associated with bone integrity.

4.9 Determination of postmenopausal bone health risk profile

- Leading risk factors for compromised bone health in the postmenopausal period are the long duration of MP and old age. According to our results, changes in bone density in postmenopausal women were determined in about 70% of these two risk factors. As a threshold value of the duration of MP, above which the risk of osteoporosis increases, we defined 12.5 years, and as a threshold value of the age above which the risk of osteoporosis increases, we defined 62.5 years. The risk age for postmenopausal women defined in our study is close to the risk age of 65 years, according to international recommendations, above which an assessment of BMD should be performed. The reduction in BMD in cases of an ovarian failure is associated with an

increased level of bone metabolism, at which the rate of bone formation is unlikely to compensate bone resorption. The threshold values of OC (5.18 ng/ml), Beta Crosslaps (0.44 ng/ml) and DPD/Cr (5.67 nmol/mmol) determined by us were associated with a decrease in bone density. Additionally, we noted that in the late postmenopausal period there was a deepening decrease in BMD due to decreasing bone formation with delayed increased bone resorption.

- Lower BW could be commented as an additional risk factor for compromised bone health in postmenopausal women, because it is associated with lower bone density and higher fracture risks. For a threshold value of BW, below which the risk of osteoporosis increases, we determined 66.5 kg. Thresholds of BW, below which fracture risks increase, were also found in this study - 62 kg for the 10-year risk of MOF and 66.5 kg for the 10-year risk of HF. Below these thresholds for BW, it could be said that the probability of fracture increases according to the 10-year risk of MOF and the 10-year risk of HF.

- On the other hand, the loss of the positive relationship between WC and BMI with BMD parameters after BW adjustment in combination with the established positive relationships between BMI and fracture risks from multifactorial stepwise analyses suggest that obesity could counteract the positive effects associated with mechanical skeletal load. Additionally, obesity was found to be associated with lower levels of bone turnover. Calculations revealed that 31% of the levels of the bone metabolic markers depend on WC.

- Following the assessment of carbohydrate metabolism in postmenopausal women without established diabetes mellitus, several additional potential risk factors have been identified. Lower basal insulin levels (fasting insulin) were associated with a higher 10-year risk of MOF because of lower BMD parameters found in cases with preserved insulin sensitivity (HOMA index < 2). Risk values of fasting insulin in relation to the 10-year risk of MOF are those below 6.15 μIU/ml. On the other hand, higher stimulated insulin levels (insulin at 120 min) were associated with a higher 10-year risk of MOF because of lower BMD parameters found in the progression of IR (HOMA index > 2). Risk values of insulin at 120 min in relation to the 10-year risk of MOF are those above 39.7 μIU/ml. On the other hand, the established relationships between the estimated bone parameters and glycemic levels in the absence of

diabetes mellitus direct attention to negative associations of fasting BG and BG at 120 min with bone integrity.

- The assessment of lipid metabolism in postmenopausal women in the present study did not highlight a specific lipid parameter as positively or negatively related to bone integrity. However, negative associations in more severe atherogenic dyslipidemia could not be ruled out.

- According to the analysis, we assumed that AH and especially its poor control is a risk factor for bone health in postmenopausal women (OR = 2.14 (0.686-6.703); p = 0.015), as it was associated with lower BMD and higher fracture risks with established negative correlation between DBP and BMD and positive relationship between DBP and fracture risks. The observed results suggest that the negative impact of AH on bone is determined not only by the values of BP, but also by the lack of timely initiated antihypertensive therapy, as well as the stage of AH.

According to the identified additional risk factors for bone health, we offer a more complex diagnostic and therapeutic algorithm with a view to timely and effective prevention, prophylaxis and treatment of osteoporosis in postmenopausal age (Fig. 84).

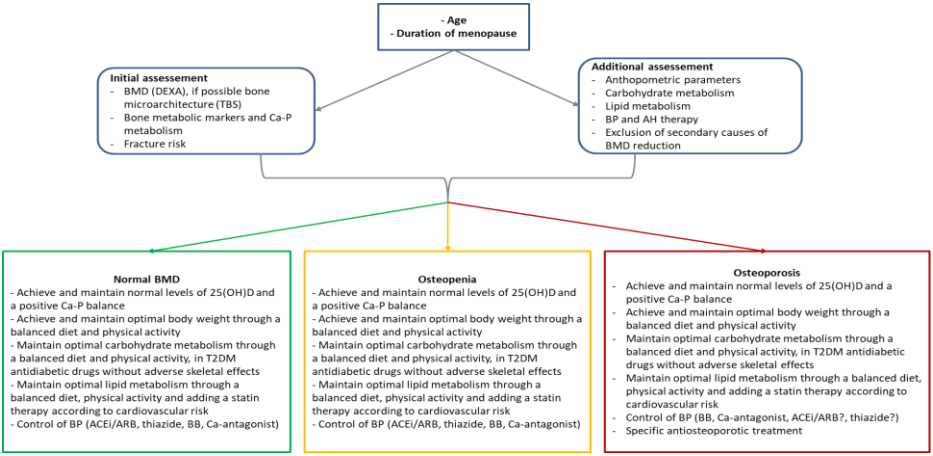

Figure 84. Bone health in postmenopausal age: diagnostic-therapeutic approach

Consequences

1. Primary postmenopausal osteoporosis is associated with an increased bone metabolism, in which bone formation falls behind bone resorption.

2. The serum bone resorption marker Beta Crosslaps is a more informative indicator of osteoclast activity, while the DPD/Cr ratio is modulated by extraosseous factors affecting glomerular filtration.

3. Age and duration of MP are leading non-modifiable risk factors for deterioration of bone health in postmenopausal age.

4. Optimal levels of 25(OH)D and calcium-phosphorus balance are preconditions for better bone integrity in postmenopausal women.

5. Body weight is associated with better parameters of bone integrity, but this relationship could be counterbalanced by obesity.

6. Higher basal insulin levels with preserved insulin sensitivity are associated with better bone integrity, while insulin resistance and concomitant hyperinsulinemia are associated with deteriorating bone health in postmenopausal age.

7. The relationship between lipid metabolism and bone health could be divergent, probably determined by the degree of deviation in different lipid parameters.

8. The proinflammatory cytokine TNF alfa negatively correlates with bone integrity, while the inflammatory marker CRP is not associated with changes in bone health in the postmenopausal period.

9. The negative association of AH with bone health in postmenopausal age is determined by the maintenance of higher DBP, the lack of timely initialed antihypertensive therapy and the stage of AH.

10. The complex relationship between metabolic syndrome and bone health depends on the number, combination and degree of manifestation of the individual components of the syndrome.

Contribution

Scientific-theoretical contributions

1. According to the studied literature, it was compared for the first time in Bulgaria the relationship between the MetS and its components with BMD, bone metabolic markers and fracture risks in postmenopausal women.
2. According to the studied literature, it was assessed for the first time in Bulgaria not only the relationship between BP with bone health in postmenopausal age, but also the associations of bone parameters with AH stage. Such analyses are also limited worldwide. In addition, the association of some antihypertensive drugs with bone integrity has been evaluated.

Scientific-practical contributions

1. In the assessment of bone metabolism, a direct comparison was made between the serum marker for bone resorption Beta Crosslaps and the urine marker for bone resorption DPD/Cr ratio. The performed analyses emphasize the higher diagnostic value of the serum marker Beta Crosslaps and point to extraosseous influences on the levels of the DPD/Cr ratio.
2. Based on the obtained results, a risk profile for compromised bone health in postmenopausal age has been determined.
3. According to the identified additional determinants of bone health in postmenopausal age, a comprehensive analysis of the cardio-metabolic profile in the assessment of bone integrity is recommended.
4. In addition, recommendations are given for non-drug and drug correction of the identified additional risk factors in order to more comprehensive prevention, prophylaxis and treatment of osteoporosis, as well as to avoid adverse skeletal effects associated with the treatment of comorbidities.

Confirmatory contributions

1. Quantitative changes in BMD in postmenopausal women are significantly associated with longer duration of MP and older age.
2. Lower body weight is associated with less favorable bone integrity.

3. The analysis of 25(OH)D levels confirmed the predominance of Vitamin D deficiency and insufficiency but also emphasized the role of optimal vitamin levels in maintaining normal BMD, as well as the importance of compensating its lower levels for the prevention of osteoporosis.

Conclusion

In fact, the onset of MP and older age are the leading non-modifiable risk factors for the development of osteoporosis in females. These data are also confirmed in our study. In addition, we reported that a decrease in BMD and a higher fracture risks in postmenopausal age were associated with a higher level of bone turnover, where the rate of bone formation could not compensate the increased bone resorption.

However, the present study also identified additional potential determinants of bone health that could help in the prevention, prophylaxis and treatment of osteoporosis if they are identified and corrected in a timely manner. Maintaining a positive Ca-P balance, for example, could be a precondition for better bone parameters in postmenopausal women. In addition, the established positive correlation between 25(OH)D and BMD in healthy controls confirms the role of the vitamin in maintaining normal BMD, as well as the importance of compensating its lower levels for the prevention of osteoporosis.

Anthropometric parameters are also related to bone parameters in postmenopausal women. Higher BW is identified as the main anthropometric factor associated with better BMD in postmenopausal age. This relationship is probably due to the greater mechanical load that the BW exerts on the skeleton. In addition, the identified negative relationships with fracture risks confirm the positive associations between postmenopausal bone health and BW. On the other hand, the loss of the positive relationship between WC and BMI with BMD parameters after BW adjustment and the lower level of bone turnover found in obesity combined with the positive dependence of BMI on fracture risk suggest that obesity could counteract the positive effects associated with the mechanical load on the skeleton.

The assessment of carbohydrate metabolism in postmenopausal women without established diabetes mellitus also points to potential additional

determinants of bone health after the onset of MP. The established negative correlation between BG at 120 min and BMD, as well as the stated positive correlation between BG at 120 min and the fracture risks suggest a negative association of higher postprandial glucose with bone integrity, especially in cases of reduced insulin sensitivity. In addition, fasting BG was positively associated with the fracture risks. On the other hand, at HOMA index > 2, insulin at 120 min was associated with lower BMD and higher fracture risk. These results suggest a negative association between postmenopausal bone health and higher stimulated insulin levels in the progression of IR. However, if insulin sensitivity was maintained, higher basal insulin levels were associated with better bone health parameters, as fasting insulin was associated with higher BMD and a lower fracture risks at HOMA index < 2. These observations suggest that the relationship between insulin and bone is modulated by the level of insulin sensitivity. Our results support the hypothesis of the osteoanabolic action of insulin, but only in the cases of preserved insulin sensitivity. On the other hand, we assumed that peripheral resistance to insulin hypoglycemic action is accompanied by insulin resistance at the skeletal level. However, the potential of OC to affect insulin sensitivity and glucose homeostasis should also be discussed.

The assessment of lipid metabolism in postmenopausal women in the present study did not highlight a specific lipid parameter as positively or negatively related to bone integrity. However, negative associations in more severe atherogenic dyslipidemia could not be ruled out. Because of the small number of women in the total group receiving antilipemic therapy, we could not comment on the relationship between postmenopausal bone health and statin intake.

According to the established negative relationship of TNF alpha with BMD parameters and the positive relationship of TNF alpha with the fracture risks, we assumed the presence of a direct negative relationship between proinflammatory cytokine and bone integrity. On the other hand, no correlations and dependencies were found between CRP and postmenopausal bone health, despite the positive association of the inflammatory marker with the HOMA index and insulin levels.

The presence of AH was also emerging as a possible additional determinant of bone health in postmenopausal women, as it was associated with lower BMD and higher fracture risks. In addition, a positive correlation of BP with urinary calcium loss and a negative association of BP with serum P levels were found. The negative relationship between bone integrity and AH was determined by the lack of initiated antihypertensive therapy, maintenance of higher DBP and the stage of AH. In addition, our data support the opinions on the potential pleotropic effects of antihypertensive drugs on bone. Based on the obtained results, we assumed that the timely initiation of antihypertensive therapy and maintaining optimal BP could help in the prevention and prophylaxis of osteoporosis, as the presumed pleotropic effects of the drugs on bone probably depend on the duration of their intake and baseline bone parameters at which antihypertensive treatment has been initiated. We believe that properly selected and timely initiated antihypertensive therapy could be useful in preventing possible fractures. In addition, it seems relevant to assess patients with AH and especially postmenopausal women for osteoporosis. After the comparative, correlation and multifactorial analyses in the observed cases according to the presence of MetS, we found that the individual components of the syndrome could be associated with divergent changes in bone integrity. In conclusion from the conducted complex analyses we assumed that the overall relationship between MetS and bone health depends on the number, combination, the degree of manifestation of the components of the syndrome, as well as the concomitant anthropometric and metabolic changes. On the other hand, we considered that it is necessary to timely identify and correct the negative parameters related to bone integrity through an appropriate diet and physical activity, as well as the addition of drugs that have not only cardiometabolic benefits but also presumed pleotropic skeletal effects.

List of publications related to the dissertation

1. Dimitrova, R, Hristozov, K. Osteoporosis - significance of the problem. Medinfo (Jubilee Edition), 2020; 1: 178-181.
2. Dimitrova, R. Bone and visceral adipose tissue - a literature review. Varna Medical Forum. 2020; 9 (2): 68-77
3. Dimitrova, R. Bone and insulin resistance - literature review. Varna Medical Forum. 2020; 9 (2): 78-88
4. Dimitrova, R., Todorov, G. Arterial hypertension and bone health - literature review. Varna Medical Forum. 2021; 10 (1): 52-63
5. Radina Dimitrova, Kiril Hristozov, Mila Boyadzhieva. Postmenopausal bone health may be influenced by the presence of arterial hypertension and antihypertensive therapy - Scripta Scientifica Medica. 2021 (online)

Scientific projects in connection with the dissertation

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