

Metabolic and bone profile in postmenopausal women with and without type 2 diabetes: a cross-sectional study

NICOLETA DUMITRU^{1,2}, MARA CARȘOTE^{1,2}, ANDRA COCOLOS^{1,2}, EUGENIA PETROVA^{1,2}, MARIA OLARU¹,
ANDRA CARAGHEORGHEOPOL¹, CONSTANTIN DUMITRACHE², ADINA GHEMIGIAN^{1,2}

¹“C.I. Parhon” National Institute of Endocrinology, Bucharest, Romania

²“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

Introduction. Current studies support the implication of metabolic changes associated with type 2 diabetes in altering bone metabolism, structure and resistance.

Objective. We conducted a cross-sectional study on postmenopausal women aimed to analyze the differences in metabolic and bone profile in patients with and without type 2 diabetes

Methods. We analyzed the metabolic and bone profile in postmenopausal women with and without type 2 diabetes (T2DM). Clinical, metabolic, hormonal parameters, along with lumbar, hip and femoral bone mineral density (BMD) and trabecular bone score (TBS) were evaluated.

Results. 56 women with T2DM (63.57±8.97 years) and 83 non-T2DM (60.21±8.77 years) were included. T2DM patients presented a higher value of body mass index (BMI) and BMD vs. control group ($p = 0.001$; $p = 0.03$ -lumbar level, $p = 0.07$ -femoral neck and $p = 0.001$ -total hip). Also, BMI correlated positively with lumbar-BMD and glycated hemoglobin (HbA1c) ($r = 0.348$, $p = 0.01$; $r = 0.269$, $p = 0.04$), correlation maintained even after age and estimated glomerular filtration rate (eGFR) adjustment ($r = 0.383$, $p = 0.005$; $r = 0.237$, $p = 0.08$). Diabetic patients recorded lower levels of 25(OH)D ($p = 0.05$), bone markers ($p \leq 0.05$) and TBS ($p = 0.07$).

For the entire patient group we found a negative correlation between HbA1c level and bone markers: $r = -0.358$, $p = 0.0005$ -osteocalcin, $r = -0.40$, $p = 0.0005$ -P1NP, $r = -0.258$, $p = 0.005$ -crosslaps.

Conclusions. Our results indicate the presence of altered bone microarchitecture in T2DM patients according to the TBS score, combined with lower levels of bone markers, with a statistically significant negative correlation between HbA1c level and bone markers.

Keywords: type 2 diabetes mellitus, diabetic bone disease, trabecular bone score, osteocalcin, bone density.

INTRODUCTION

The latest studies have established the active character of bone tissue, with important implications for the general homeostasis of the organism. It is now increasingly evident the role as endocrine organ exerted by the skeletal system, proving complex links with adipose tissue and glycaemic and energy metabolism [1, 2]. Thus, osteocalcin secreted by osteoblast, in its undercarboxylated form, functions as a hormone with a role in regulating secretion and sensitivity to insulin, as well as energy consumption [1, 3-6].

On the other hand, insulin, insulin-like growth factor type 1 (IGF1) and incretin hormones have a role in regulating bone homeostasis [2]. The binding of insulin to specific receptors on osteoblast exerts anabolic effects, favoring survival, proliferation and differentiation of osteoblasts [3, 8]. More importantly, however, is the mechanism for promoting bone resorption insulin-induced by decreasing the

expression of osteoprotegerin (OPG) in the osteoblast favoring the osteoclasts function [1, 7, 8]. During bone resorption, osteoclasts generate an acidic pH at resorption lacunae level, making it a suitable environment for stored osteocalcin decarboxylation in the bone matrix, allowing it to enter the systemic circulation [3, 4].

This complex relationship bone tissue – insulin – glycaemic metabolism can be disrupted in pathological conditions, such as type 2 diabetes mellitus (T2DM) [1]. This chronic condition is characterized by progressive loss of insulin secretion by beta-pancreatic cells, in the context of peripheral insulin resistance, with impaired glucose utilization at tissue level and, consequently, with the occurrence of chronic hyperglycemia [1, 9]. Current studies support the involvement of metabolic changes associated with T2DM in bone deterioration in terms of metabolism, structure and resistance. Chronic hyperglycemia affects both the bone cell component and the extracellular matrix by accumulating the

final products of advanced glycation (AGEs) [1, 2, 10-12]. This is responsible for collagen bone matrix stiffening, that would allow the formation and propagation of micro-cracks throughout bone matrix [11]. Also, AGEs favors generation of reactive oxygen species within the bone, inducing an increase of inflammatory cytokines, that result in osteoblast dysfunction and osteoclast activation [2, 13]. Diabetic bone disease is currently being considered a complication of diabetes mellitus, characterized by compromising osteoblasts function, but also by defects in microarchitecture and bone resistance, with a final effect of increasing the risk of fragility fracture [14]. According to the ROTTERDAM study, patients with poorly controlled T2DM ($\text{HbA1c} \geq 7.5\%$) have a general risk of fracture by approximately 70% higher than non-diabetic subjects [1, 2, 16, 17].

The purpose of this study is to analyze the influence and relation between clinical, metabolic and hormonal parameters and bone profile in patients with and without type 2 diabetes.

MATERIAL AND METHODS

We conducted a cross-sectional study over a period of 27 months (January 2016 – March 2018) at “C.I. Parhon” National Institute of Endocrinology. A total of 139 postmenopausal women with and without T2DM were included in the study. Clinical features: age, weight, height, body mass index (BMI) calculated as weight divided by height squared (kilograms per square meter), were recorded. Biochemical and hormonal determinations have been made from peripheral venous blood. Thus, by absorption spectrophotometry it was established the level of serum glucose and creatinine. The level of glycated hemoglobin (HbA1c) was measured by immunoturbidimetric assay, using the Cobas C 501 analyser (Roche Hitachi Corporation Japan). The CKD-EPI creatinine Equation (2009) was used to calculate eGFR (www.kidney.org/gfr_calculator). Parathyroid hormone level (PTH), 25 hydroxy vitamin D (25 (OH) D) and bone markers: crosslaps – bone resorption marker; osteocalcin and N-terminal propeptide of type I procollagen (P1NP) – bone formation markers were determined by electrochemiluminescent immunoassay (Cobas e 601 C, Roche Hitachi Corporation Japan).

Exclusion criteria were: cirrhosis, reduced glomerular filtration rate ($\text{GFR} < 45 \text{ mL/min/1.73 m}^2$), hyperthyroidism, primary hyperparathyroidism, Cushing’s syndrome (exogenous or endogenous: pituitary, adenal or paraneoplastic), acromegaly,

rheumathoid arthritis, celiac disease, prior bone pathology (multiple myeloma, Paget’s disease) or anti-osteoporotic drugs exposure, active neoplasms, type 1 diabetes or secondary causes of diabetes. An informed consent was obtained from the study participants.

STATISTICAL ANALYSIS

All the parameters were centralized in an Excel file used for data analysis. Data were expressed as mean and standard deviation or as number/percent. We analyzed variables with a Student t test (two-tailed). Correlation analysis using Spearman’s correlation and partial correlation coefficients on specific covariates controlling for age and eGFR was performed with IBM SPSS software. Statistical significance was considered at $p < 0.05$.

RESULTS

56 women known with T2DM (63.57 ± 8.97 years) and 83 women without T2DM (60.21 ± 8.77 years) were included. Among T2DM patients, the analysis of clinical and laboratory parameters showed higher BMI values ($p = 0.001$) and glycemic control indices: fasting plasma glucose and HbA1c ($p = 0.0005$), significantly lower levels of eGFR ($p = 0.02$), 25 (OH) D ($p = 0.05$) and bone markers ($p \leq 0.05$) (Table 1). DXA evaluation revealed the presence of an increased BMD at all three sites analyzed (lumbar, femoral neck, total hip) in the group of diabetic patients, but with a lower TBS score than the control group (Figures 1 and 2).

In the group of patients with T2DM, correlation analysis showed a moderate, significant positive association between BMI and lumbar BMD ($r = 0.348$, $p = 0.009$) (Figure 3) and a poorly positive correlation between BMI and HbA1c ($r = 0.257$, $p = 0.058$), at the limit of statistical significance, maintained even after partial correlation adjustment for age and eGFR ($r = 0.383$, $p = 0.005$; respectively $r = 0.269$, $p = 0.047$). A poorly positive correlation with borderline significance between BMI-femoral cervical BMD ($r = 0.246$, $p = 0.071$) was also outlined.

The analysis also indicated, in the T2DM group, statistically significant negative correlations between BMI and the bone resorption marker: crosslaps ($r = -0.351$, $p = 0.009$) (Figure 4) and negative correlations between lumbar BMD and osteocalcin ($r = -0.334$, $p = 0.01$), respectively crosslaps ($r = -0.346$, $p = 0.009$). These negative relationships between BMI and BMD on the one

side and bone markers on the other side are maintained even after partial correlation adjusting for age and eGFR: $r = -0.466$, $p < 0.05$ for the BMI-crosslaps correlation; $r = -0.383$, $p = 0.005$ for lumbar BMD-osteocalcin correlation, respectively $r = -0.477$, $p < 0.05$ for the correlation of lumbar BMD-crosslaps.

Regarding the association of glyceimic metabolism-bone metabolism, our study indicated the presence of a negative correlation between HbA1c level and bone markers for the whole patients group: $r = -0.358$, $p = 0.0005$ for osteocalcin, $r = -0.400$, $p = 0.0005$ for P1NP, respectively $r = -0.258$, $p = 0.005$ for Crosslaps (Figure 5).

Table 1
Clinical, metabolic and hormonal parameters in the two studied groups

Parameters	T2DM Group		Control Group		P value
	Mean	SD [□]	Mean	SD [□]	
Age (years)	63.57	8.97	60.21	8.77	0.003
BMI (kg/m ²)	31.04	4.55	27.59	4.73	0.001
FPG (mg/dL)	135.81	40.30	91.99	9.73	0.0005
HbA1c (%)	6.84	1.24	5.47	0.31	0.0005
eGFR (mL/min/1.73 m ²)	84.03	20.55	90.20	13.35	0.025
25(OH)D (ng/mL)	18.51	8.44	21.98	9.53	0.05
PTH (pg/mL)	46.63	16.88	51.27	17.18	0.277
Osteocalcin (ng/mL)	18.70	7.41	24.96	8.12	0.001
P1NP (ng/mL)	41.95	15.50	58.23	30.09	0.005
Crosslaps (ng/mL)	0.375	0.178	0.484	0.213	0.041

□ SD: standard deviation

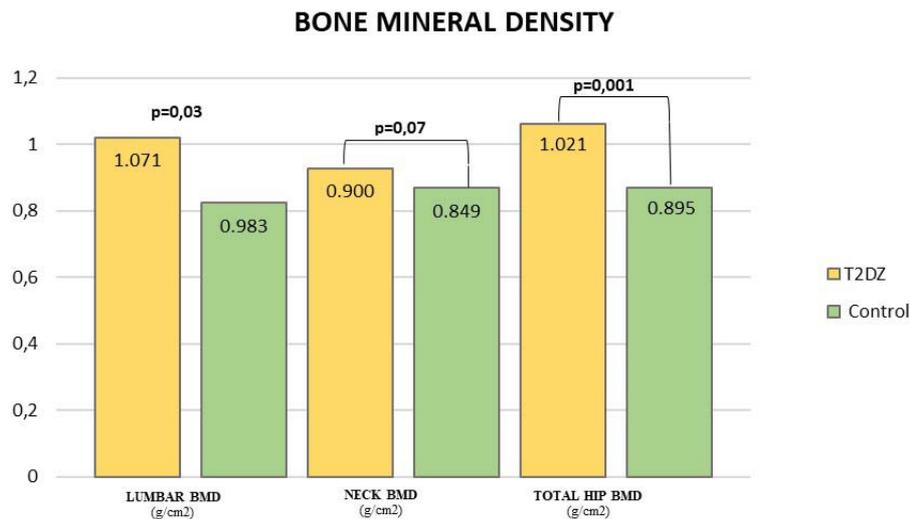


Figure 1. BMD in the studied groups.

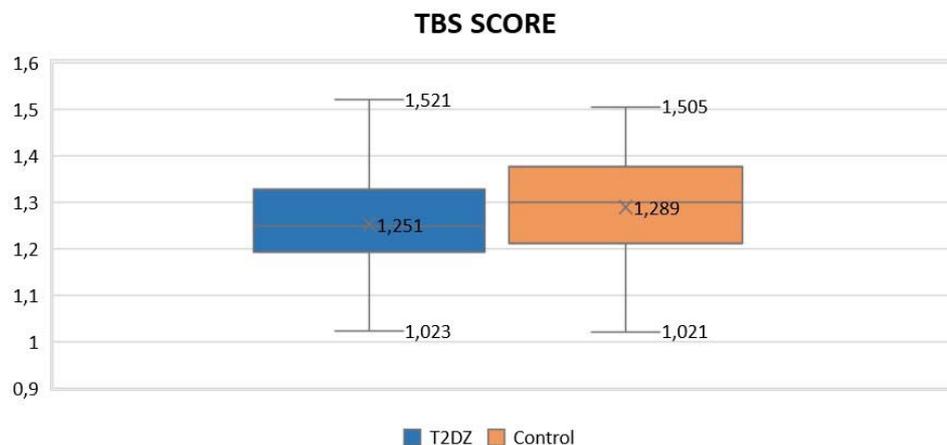


Figure 2. TBS Trabecular Bone Score in studied groups.

BMI -LUMBAR BMD CORRELATION IN T2DM GROUP

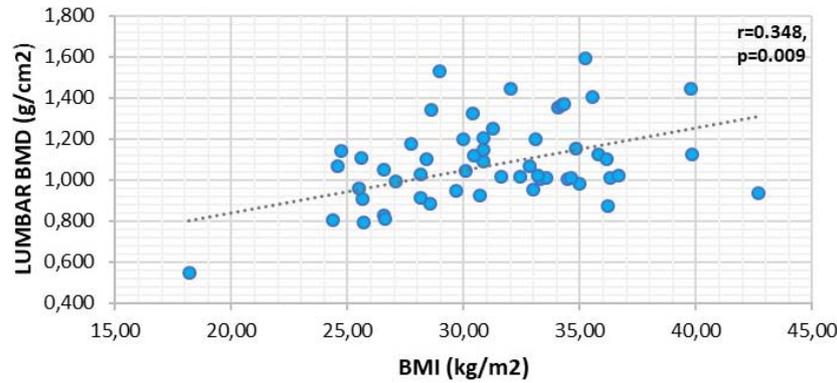


Figure 3. BMI-lumbar BMD correlation in the group of diabetic patients.

BMI-BONE RESORPTION CORRELATION IN T2DM GROUP

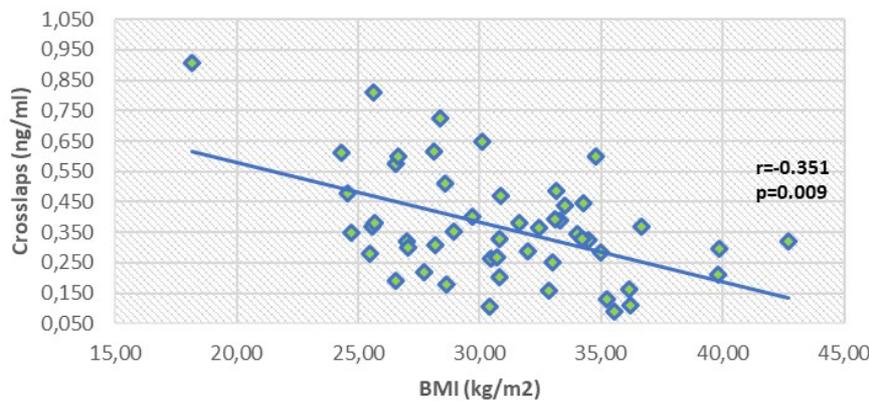


Figure 4. BMI- bone resorption correlation in the group of diabetic patients.

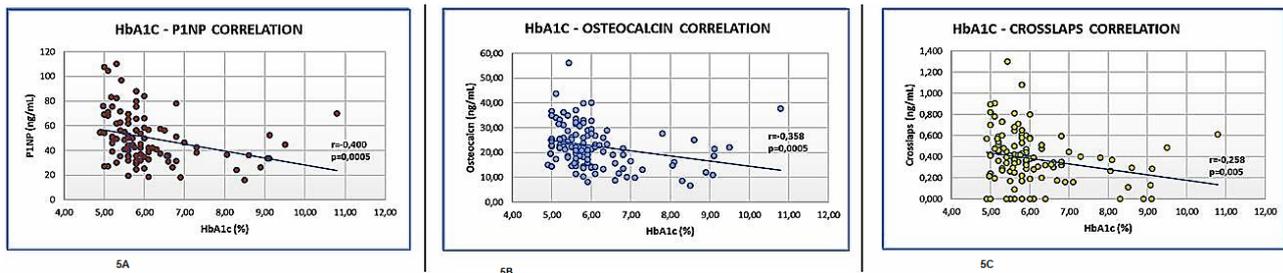


Figure 5. Correlation between HbA1c and PINP (5A), Osteocalcin (5B) and crosslaps (5C).

DISCUSSION

In our study, 60.71% of diabetic patients fell into the category of obese people based on BMI compared to only 30.12% of patients in the control group. Thus, weight excess is a strong clinical feature associated with T2DM, fact supported also by WHO report on diabetes [22]. The weight excess leads to bone mass adjustment to the degree

of mechanical load [10, 12, 23], mechanism confirmed by the presence of a positive correlation between BMI and BMD in the T2DM patient group. However, overweight and obese patients present an increased risk of fracture to minimal trauma, such as proximal humerus fracture or ankle [10, 24], thus an increased BMI is no longer a protective factor against fragility fractures as it was considered in the past [5, 12, 23, 24]. Thereby, the

risk of fracture in diabetic patients is less dependent on the bone quantity (bone mass density), but more on bone quality [12, 26, 27].

Trabecular microarchitecture is an important component of bone quality next to: bone matrix, mineral composition, bone turnover and bone geometry [11]. We can evaluate bone microarchitecture by using the trabecular bone score (TBS), the newest clinical tool for bone microarchitecture damage assessment. This method allows indirect evaluation of trabecular structure through software analysis of lumbar spine image obtained at osteodensitometric evaluation [20, 21]. A value of TBS > 1.350 indicates a normal trabecular structure, while a value below 1.350 corresponds to trabecular deterioration, which favors occurrence of fragility fractures [14]. In the study group, more than three quarters of diabetic patients (n = 44; 83%) presented a TBS value < 1.350, despite the association of a significantly increased lumbar BMD compared with the control group. Of these, 7 (15.9%) had a severely degraded microarchitecture, with a TBS value < 1.100. In the non-diabetic group, 51 patients (69.86%) have degraded trabecular microarchitecture, of which only 4 (7.84%) presented a TBS < 1.100.

Bone turnover, the complex process between bone formation and resorption, has an important role in determining bone quality [23]. Clinically, this process can be evaluated by bone markers analysis from the serum, plasma or urine [28, 29]. In daily practice, the markers commonly used are: osteocalcin – element of the bone matrix, P1NP – precursor of type 1 collagen and crosslaps, type 1 collagen degradation product. Numerous studies indicate the presence of a low bone turnover in patients with T2DM [5, 12, 24, 29]. This condition allows accumulation of micro-fractures in the bone, with altered bone quality and increased bone fragility, independent of BMD [5, 29, 30]. Multiple mechanisms are involved in the reduction of bone resorption and formation in type 2 diabetes, such as: hyperglycemia and insulin resistance, but also obesity and metabolic syndrome associated with diabetes, that tend to suppress bone turnover [24, 28-30]. The influence of these mechanisms explains the presence in our study of negative correlations between BMI and BMD on the one side and bone markers on the other side in diabetic patients. This highlights the decoupling of bone turnover from bone mineralization, which remains intact [5, 29], that generates a normal or higher BMD than age-estimated in these patients.

Our analysis also indicated the presence of negative correlations between HbA1c levels and turnover markers, both forming (osteocalcin, P1NP), as well as bone resorption (crosslaps), independent of T2DM presence. This may be explained in part by the fact that diabetes patients did not fit in the category of poor glycemic control with a mean HbA1c of $6.84 \pm 1.24\%$. Other limitations of our study are the fact we did not consider duration of diabetes or the antidiabetic medication used. Also, it would have been useful to dose the serum insulin level and calculate the insulin resistance index (HOMA-IR). This could provide additional information on the complex relationship between bone – adipose tissue – glycemic metabolism, that can be disrupted by the presence of type 2 diabetes mellitus.

Our study results want to highlight the importance of bone quality in type 2 diabetes patients, with a major impact on the fracture risk in these patients. The impairment of diabetic bone quality is not underlined by bone mineral density determination, the gold standard for diagnosis of osteoporosis. Also, the FRAX tool that calculates the 10-year probability of fracture based on clinical factors and femoral neck bone mineral density, underestimates this risk in patients with T2DM [13, 15-17]. A more efficient method of estimating the risk in these patients is represented by the adjustment of the FRAX score with the value of the Trabecular Bone Score [17].

CONCLUSION

Diabetic bone disease is a complex metabolic disorder characterized mainly by bone quality deterioration. The decrease in bone turnover and deterioration of trabecular microarchitecture, elements that characterize the bone in qualitative terms, are involved in increasing bone fragility in diabetic patients and are favored by the metabolic imbalance. Given the increase in life expectancy, as well as of the type 2 diabetes prevalence, diabetic bone disease is becoming increasingly important in clinical practice. The importance of this condition lies both in terms of reducing the quality of life for these patients, but especially on the increased risk of mortality associated with hip fracture.

Acknowledgements. We thank Mr Morel Stoiceanu, main medical assistant in Radiology laboratory, for helping us with osteodensitometric evaluation of the patients included in the study.

Conflict of interest. The authors declare that there are not conflicts of interest.

Introducere. Studiile actuale susțin implicarea modificărilor metabolice asociate diabetului zaharat tip 2 în alterarea metabolismului, structurii și rezistenței osoase.

Obiectiv. Am realizat un studiu transversal pe un grup de femei în postmenopauză, cu scopul evaluării diferențelor privind profilul metabolic și osos la paciențe cu și fără diabet zaharat tip 2.

Metodă. Am evaluat parametrii clinici, metabolici, hormonal, alături de densitatea minerală osoasă (DMO) de la nivel lombar, șold total, col femural și a scorului de os trabecular (TBS).

Rezultate. Au fost incluse 56 paciențe cu DZ2 (63.57 ± 8.97 ani) și 83 femei (60.21 ± 8.77 ani) în lotul martor. Paciențele DZ2 au prezentat o valoare mai mare a IMC și a DMO vs lotul martor ($p = 0.001$; $p = 0.03$ – nivel lombar, $p = 0.07$ – col femural, $p = 0.001$ – șold total). Iar IMC-ul s-a corelat pozitiv cu DMO-lombară și cu nivelul hemoglobinei glicate (HbA1c) ($r = 0.348$, $p = 0.01$; $r = 0.269$, $p = 0.04$), menținut și după ajustare pentru vârstă și eRFG ($r = 0.383$, $p = 0.005$; $r = 0.237$, $p = 0.08$). De asemenea, paciențele diabetice au înregistrat nivele mai mici ale 25(OH)D ($p = 0.05$), ale markerilor osoși ($p \leq 0.05$) și a TBS ($p = 0.07$). Pentru întreg grupul de paciențe s-a înregistrat o corelație negativă între nivelul HbA1c și markerii osoși: $r = -0.358$, $p = 0.0005$ – osteocalcină; $r = -0.40$, $p = 0.0005$ – PINP; $r = -0.258$, $p = 0.005$ – crosslaps.

Concluzii. Rezultatele noastre indică prezența unei microarhitecturi osoase deteriorate la paciențele cu DZ2 conform scorului TBS, asociată cu nivele scăzute ale markerilor osoși, cu prezența unei corelații negative, semnificativă statistic, între nivelul HbA1c și markerii osoși.

Correspondence to: Andra Cocolos, MD, “C.I. Parhon” National Institute of Endocrinology, Aviatorilor Ave 34-38, Bucharest, sector 1, postal code 011684, Romania
Tel: +40762278589
E-mail: andra_buruiana@yahoo.com

REFERENCES

- LIU JIAN-MIN, ROSEN J. CLIFFORD, DUCY PATRICIA, KOUSTENI STAVROULA, KARSENTY GERARD. *Regulation of glucose handling by the skeleton: insights from mouse and human studies*. Diabetes (2016); **65**:3225–3232.
- SANCHES CP, VIANNA AGD, BARRETO F DE C. *The impact of type 2 diabetes on bone metabolism*. Diabetology & Metabolic Syndrome. 2017;**9**:85.
- CLEMENS, T. L., KARSENTY, G. *The osteoblast: An insulin target cell controlling glucose homeostasis*. J Bone Miner Res, (2011), **26**:677-680.
- FERRON M, WEI J, YOSHIZAWA T, et al. *Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism*. Cell. 2010; **142**(2): 296-308.
- MATTHEW P. GILBERT, RICHARD E. PRATLEY. *The impact of diabetes and diabetes medications on bone health*. Endocrine Reviews, (2015);**36**(2):194–213.
- ZOCH ML, CLEMENS TL, RIDDLE RC. *New insights into the biology of osteocalcin*. Bone. 2016;**82**:42-49.
- FAIENZA MF, LUCE V, VENTURA A, et al. *Skeleton and glucose metabolism: a bone-pancreas loop*. International Journal of Endocrinology (2015); **2015**:758148.
- SHAO J, WANG Z, YANG T, YING H, ZHANG Y, LIU S. *Bone regulates glucose metabolism as an endocrine organ through osteocalcin*. International Journal of Endocrinology. 2015;**2015**:967673.
- AMERICAN DIABETES ASSOCIATION. 2. *Classification and diagnosis of diabetes: Standards of medical care in diabetes – 2018*. Diabetes Care 2018;**41**(Suppl. 1):S13-S27.
- COMPSTON J. *Type 2 diabetes mellitus and bone* (Review). J Intern Med 2018; **283**: 140-153.
- DUMITRU N., COCOLOS A., CARAGHEORGHEOPOL A., DUMITRACHE C., BRATU O.G, NEAGU T.P, DIACONU C.C, GHEMIGIAN A. *Collagen – the ultrastructural element of the bone matrix*. Rev.Chim. (Bucharest), 2018, **69**(No. 7), 1706-1709.
- MOREIRA C.A., BARRETO, F.C., DEMPSTER, D.W. *New insights on diabetes and bone metabolism*. Brazilian Journal of Nephrology, (2015) **37**(4): 490-495.
- CARNEVALE, V., ROMAGNOLI E., D'ERASMO L., D'ERASMO E. *Bone damage in type 2 diabetes mellitus*. Nutrition, Metabolism and Cardiovascular Diseases. 2014;**24** (11): 1151-1157.

14. JACKULIAK P.; PAYER J. *Osteoporosis, Fractures, and Diabetes*. *International Journal of Endocrinology*, vol. 2014, Article ID 820615, 10 p., 2014.
15. DEDE A.D., TOURNIS S., DONTAS I., TROVAS G. *Type 2 diabetes mellitus and fracture risk*. *Metabolism – Clinical and Experimental*, 2014, **63**(12): 1480-1490.
16. OEI L, ZILLIKENS MC, DEGHAN A, *et al*. *High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam study*. *Diabetes Care*. 2013;**36**(6):1619-1628.
17. RUBIN M.R. *Skeletal fragility in diabetes*. *Ann. N.Y. Acad. Sci.* 2017, **1402**: 18-30.
18. KARIM L, BOUXSEIN ML. *Effect of type 2 diabetes-related non-enzymatic glycation on bone biomechanical properties*. *Bone*. 2016;**82**:21-27.
19. SUNDARARAGHAVAN V, MAZUR MM, EVANS B, LIU J, EBRAHEIM NA. *Diabetes and bone health: latest evidence and clinical implications*. *Therapeutic Advances in Musculoskeletal Disease*. (2017);**9**(3):67-74.
20. DUMITRU N., CARSONE M., COCOLOS A., GHEMIGIAN A. *Glucose metabolism indices and Trabecular Bone Score in type 2 diabetes mellitus women*. 4th International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complication, INTERDIAB Conference Proceedings Volume, 2018, 117-125.
21. SILVA B.C, BROY S.B, BOUTROY S., SCHOUSBOE J.T., SHEPHERD J.A., LESLIE W.D. *Fracture risk prediction by non-BMD DXA measures: the 2015 ISCD Official Positions Part 2: Trabecular Bone Score*. *J Clin Densitom.* 2015;**18**(3):309-330.
22. WORLD HEALTH ORGANISATION. *Global Report on Diabetes*. World Health Organisation, 2016.
23. KREMER R., GILSANZ V. *Fat and bone: an odd couple*. *Frontiers in Endocrinology*. 2016;**6**:190.
24. WALSH J.S, VILACA T. *Obesity, type 2 diabetes and bone in adults*. *Calcified Tissue International*. 2017;**100**(5):528-535.
25. MOSELEY KF. *Type 2 diabetes and bone fractures*. *Current opinion in endocrinology, diabetes, and obesity*. 2012;**19**(2):128-135.
26. IPPEI KANAZAWA. *Interaction between bone and glucose metabolism* [Review]. *Endocrine Journal* 2017; **64**(11): 1043-1053.
27. YONG JUN CHOI, SO YOUNG OCK, YOON-SOK CHUNG. *Trabecular bone score (TBS) and TBS-adjusted fracture risk assessment tool are potential supplementary tools for the discrimination of morphometric vertebral fractures in postmenopausal women with type 2 diabetes*. *Journal of Clinical Densitometry: Assessment & Management of Musculoskeletal Health*, 2016; **19** (4): 507-514.
28. STARUP-LINDE J., VESTERGAARD P. *Biochemical bone turnover markers in diabetes mellitus – a systematic review*. *Bone* 2016;**82**:69-78.
29. HYGUM K., STARUP-LINDE J., HARSLØF T., VESTERGAARD P., LANGDAHL B.L. *Mechanisms in endocrinology: diabetes mellitus, a state of low bone turnover – a systematic review and meta-analysis*. *Eur. J. Endocrinol.* 2017;**176**:R137-R157.
30. VIANNA A.G.D, SNACHES C.P, BARRETO F.C. *Review article: effects of type 2 diabetes therapies on bone metabolism*. *Diabetology & Metabolic Syndrome*. 2017;**9**:75.

Received September 30, 2018