

The Link Between Bone Osteocalcin and Energy Metabolism in a Group of Postmenopausal Women

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

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

²"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT: There is a dual relationship between bone and tissues involved in energy metabolism (fat tissue and beta-pancreatic cells). Thus, bone remodeling is an energy consuming process, but osteocalcin, the main non-collagenic protein, synthesized by osteoblasts during bone formation exerts a number of biological effects on beta-pancreatic and adipose cells. With this data, we wanted to see if the presence of a chronic metabolic disorder such as type 2 diabetes mellitus (T2DM) influence this complex dual relationship. For this, we conducted a cross-sectional study to evaluate the relation between osteocalcin and energetic metabolism in a group of 146 postmenopausal women with and without T2DM at CI Parhon National Institute of Endocrinology, Bucharest. Clinical, metabolic and hormonal parameters were evaluated. For statistical analysis we used Student t-test and the Spearman correlation (statistical significance: $p < 0.05$). Results: 63 patients with T2DM (63.88 ± 8.56 years) and 83 women in the control group (60.21 ± 8.77 years) were included. Diabetic women showed a lower level of serum total osteocalcin ($p < 0.05$) HDL-cholesterol ($p = 0.02$), and 25-hydroxyvitamin D (25(OH)D). The body mass index (BMI), glycemic metabolism parameters and triglyceride levels ($p < 0.05$) were higher in this group. We found correlations between osteocalcin and metabolic elements: negative with BMI ($r = -0.329$, $p < 0.05$), glycated hemoglobin (HbA1c) ($r = -0.398$, $p < 0.05$), and serum triglycerides ($r = -0.329$, $p < 0.05$) respectively positive with HDL-cholesterol ($r = 0.279$, $p = 0.001$) for the entire group of patients. Conclusions: Our study indicated the presence of significant correlations between serum osteocalcin and glycemic and lipid metabolism parameters, independent of the presence of diabetes.

KEYWORDS: Bone turnover, osteoblast, osteocalcin, energy metabolism, insulin

Introduction

Bone remodeling is a physiological process essential to maintain bone mechanical qualities (resistance to fracture) and mineral homeostasis.

It involves a highly coordinated action between osteoclasts, osteoblasts and osteocytes [1].

Bone remodeling is the morphological basis of bone turnover and includes two components: resorption and bone formation.

These components are sequential, coupled and equivalent, providing the bone's ability to renew by replacing the old bone, eliminating microfractures, and adjusting bone microarchitecture to the degree of loading [2,3].

This metabolic activity is energy-consuming, making bone remodeling a process highly dependent on the energy status of the body [4,5,6].

This addiction triggered the identification of hormonal factors involved in the control of bone remodeling, but also of energy metabolism [5].

Thus, studies have established that osteocalcin, the main non-collagenic protein of the bone matrix synthesized by osteoblast, is involved in regulating energy metabolism

through effects on beta-pancreatic cells and adipocytes.

In this way it coordinates the two processes, providing the energy needed for the bone remodeling process [6,7].

Osteocalcin or the bone-Gla protein, named due to the presence of three γ -carboxyglutamic acid residues, which allows it to bind to hydroxyapatite crystals in bone matrix, is also present in systemic circulation as total or partially carboxylated and uncarboxylated forms [2,5,6].

The last is approximately 40-60% of the total seric osteocalcin.

Identification of the osteocalcin membrane receptor GPRC6A, expressed in different tissues as: skeletal muscle, pancreatic β -cells, testis, liver, has led to establishment of osteocalcin hormonal role [5].

The binding of uncarboxylated osteocalcin to GPRC6A induce pancreatic β -cells proliferation and increase insulin synthesis and secretion.

Furthermore, osteocalcin enhances energy expenditure and insulin sensitivity by increasing mitochondrial biogenesis in skeletal muscles and adiponectin expression in fat cells and decreasing lipid accumulation in liver [5,8].

On the other hand, insulin is an important regulator of osteocalcin secretion.

Its binding to osteoblast receptor increase the level of uncarboxylated osteocalcin by promoting osteocalcin gene expression, but mostly, it reduces osteoprotegerin synthesis with consecutive activation of osteoclast bone resorption [9,10].

The low pH environment at the resorption lacunae level allows osteocalcin decarboxylation and its release from bone matrix in the systemic circulation [10].

Chronic hyperglycaemia alter the bone matrix quality by excessive accumulation of advanced glycation end products (AGEs) cross-links in collagen fibers, making it more brittle [12].

This metabolic disorder affects also the bone turnover, which will ultimately determine an increased risk for fragility fractures [10,12].

Based on these data, we aimed to evaluate the relation between serum total osteocalcin levels and metabolic and hormonal parameters in postmenopausal women with and without T2DM.

Material and Methods

We conducted a cross-sectional study over a period of 30 months (January 2016-June 2018) at C.I. Parhon National Institute of Endocrinology.

A total of 146 postmenopausal women with and without T2DM were included in the study.

Clinical parameters such as: age, weight, height, 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, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and creatinine.

The level of 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 estimated

glomerular filtration rate (eGFR) (www.kidney.org/gfr_calculator).

Hormonal measurements included: parathyroid hormone (PTH), 25(OH)D and total osteocalcin levels, and were determined by electrochemiluminescent immunoassay (Cobas e 601 C, Roche Hitachi Corporation, Japan).

Exclusion criteria were: cirrhosis, reduced glomerular filtration rate (eGFR <45mL/min/1.73m²), hyperthyroidism, primary hyperparathyroidism, Cushing's syndrome (exogenous or endogenous: pituitary, adrenal or paraneoplastic), acromegaly, rheumatoid arthritis, celiac disease, prior bone pathology or anti-osteoporotic drugs exposure, active neoplasms, type 1 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).

For correlation analysis we used Spearman's correlation (IBM SPSS software).

Statistical significance was considered at $p < 0.05$.

Results

Our study included 63 women known with T2DM (63.88±8.56 years) and 83 women without T2DM (60.21±8.77 years).

From the clinical point of view, 58 women (92,06%) in the diabetes group are classified as overweight or obese based on their BMI, compared to only 57 women (68,67%) in the control group.

Analysis of biochemical and hormonal parameters showed lower levels of HDL-cholesterol ($p=0.02$), eGFR ($p=0.02$), 25(OH)D ($p=0.04$) and osteocalcin ($p<0.05$), with higher fasting plasma glucose, HbA1c ($p<0,05$) and triglycerides values ($p=0,001$) in T2DM group versus control group. (Table 1)

Table 1. Metabolic and hormonal characteristics in the studied group

Parameters	Diabetes group	Control group	P value
Fasting glucose (mg/dL)	134,67±38,69	91,95±9,68	<0,05
HbA1c (%)	6,77±1,18	5,44±0,31	<0,05
Total Cholesterol (mg/dL)	200,57±44,09	213,70±45,71	0,15
LDL-Cholesterol (mg/dL)	120,16±40,82	134,43±45,43	0,09
HDL-Cholesterol (mg/dL)	52,10±15,03	59,83±15,99	0,02
Triglycerides (mg/dL)	147,50±72,19	108,02±44,60	0,001
eGFR (mL/min/1,73m ²)	84,03±20,50	90,20±13,35	0,02
Osteocalcin (ng/mL)	18,71±7,32	24,95±8,07	<0,05
25OHvitaminaD (ng/mL)	8,59±8,19	21,88±9,61	0,04
PTH (pg/mL)	46.63±16,88	51,27±17,18	0,27

We analysed the correlation between serum total osteocalcin and a series of clinical, biochemical and hormonal variables in the study population.

For the entire group of patients, we find a significant inverse relation between osteocalcin and BMI ($r=-0.329$, $p<0.05$).

Also, regarding metabolic parameters, osteocalcin correlated negatively with glycemic control: HbA1c ($r=-0.398$, $p<0.05$) and triglycerides levels ($r=-0.329$, $p<0.05$), and positively with HDL-cholesterol ($r=0.279$, $p=0.001$). (Fig.1)

We performed correlation analysis separately in the two groups studied to see any differences.

In the T2DM group of patients with diabetes we have not registered significant correlations between osteocalcin and BMI ($r=-0.162$, $p=0.2$) or any association with metabolic parameters: HbA1c ($r=-0.208$, $p=0.1$), triglycerides levels ($r=-0.153$, $p=0.2$) and HDL-cholesterol ($r=0.104$, $p=0.4$).

In the control group we found a significant negative correlation with triglycerides levels ($r=-0.390$, $p<0.05$) and a trend for positive association with HDL-cholesterol ($r=0.201$, $p=0.09$), with borderline significance.

Also, in this group, there was no association between osteocalcin and BMI ($r=-0.132$, $p=0.2$), respectively with HbA1c ($r=0.008$, $p=0.9$).

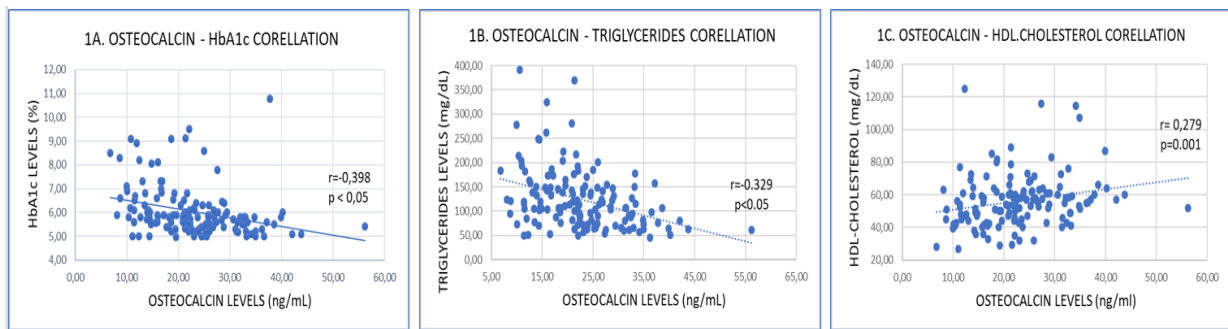


Fig.1. Correlation between osteocalcin and metabolic parameters: HbA1c (1A), Triglycerides (1B), HDL-cholesterol (1C) for the entire group of patients

The analysis of the osteocalcin correlation with other covariates, showed us a positive association with 25OHD levels ($r=0,220$, $p<0,05$) for the entire group of patients. (Fig.2).

This weak positive relationship was also present at subgroups analysis: $r=0,190$, $p=0,04$ in T2DM women and $r=0,200$, $p=0,01$ in control group.

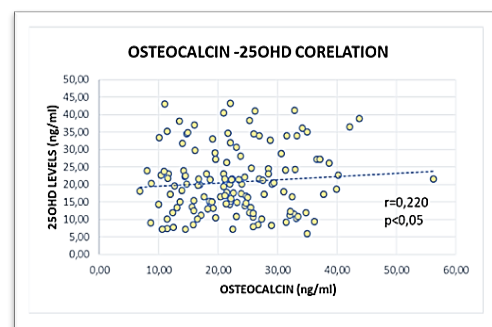


Fig.2. Association between osteocalcin and 25(OH) D levels in the study group

Discussions

Our study indicated the presence of significant associations between total serum osteocalcin and metabolic syndrome elements, independent of T2DM presence.

The association between obesity, dyslipidemia, hypertension and insulin resistance underlies the metabolic syndrome definition [11].

Among this features, central obesity plays an important role in metabolic syndrome, being associated with insulin resistance.

Together these contribute to the generation of metabolic imbalance [11].

Also, body weight is positively associated with bone mass density, and adiponectin, respectively leptin controls the secretion of osteocalcin [5,10].

But central obesity tends to suppress bone turnover, and is negatively correlated with serum osteocalcin in type 2 diabetes [10].

Thus, there is a complex relationship between adipose tissue-bone-glycemic metabolism [10].

The major utility of metabolic syndrome in clinical practice is to predict the risk of developing type 2 diabetes along with the cardiovascular disease risk, especially in the group of patients with metabolic syndrome and impaired fasting glucose [11,13].

So, type 2 diabetes mellitus should be considered a major outcome in the evolution of metabolic syndrome [11].

Our study findings are in agreement with many other clinical and epidemiological studies and meta-analysis of observational studies that showed lower total osteocalcin levels in adults with T2DM or metabolic syndrome, that were associated with higher BMI, plasma glucose values and lower insulin sensitivity [4,7,14,15,16].

Our study limits are the fact we did not consider the age of diabetes or antidiabetic therapy used, and the diabetic patients do not fall into the category of poorly controlled diabetes.

The mean value for HbA1c in our group is $6,78 \pm 1,18\%$.

Although the undercarboxylated osteocalcin is the active form of osteocalcin capable of fulfilling its endocrine function, there is a lack of concordance among studies in terms of the best variant (undercarboxylated, carboxylated and total osteocalcin) that is associated with glycometabolic status [4,17].

This is in part related of current limitations of undercarboxylated osteocalcin determination

due to lack of standardized and systematized measurement methods [7,17].

Moreover, the level of undercarboxylated osteocalcin is highly dependent of the vitamin K status [4,7,17].

Another element involved in the positive control of osteocalcin synthesis is vitamin D.

The level of 25(OH)D was less than 30ng/mL, considered optimal, in both diabetes and control group.

Vitamin D deficiency is common in postmenopausal women, and also the android fat disposition, a hallmark of metabolic syndrome is negatively associated with 25(OH)D levels [18].

Our study showed a significant positive correlation between serum total osteocalcin and 25(OH)D for the entire patient group (Fig.2).

In humans, the transcription of bone gamma-carboxyglutamic acid protein gene (*BGLAP*), located on chromosome 1 (1q25-q31), encoding osteocalcin is positively regulated by vitamin D favoring gene transcription through vitamin D responsive elements [17].

In conclusion, our study wishes to highlight the clinical value of serum osteocalcin, which is not just a simple marker of bone formation.

This is a major component in the complex relationship between bone and energy metabolism involved tissues, such as pancreatic beta cells and adipose tissue.

Osteocalcin acts as a hormone regulating secretion and insulin sensitivity.

For this reason, diseases associated with metabolic disorder such as obesity, metabolic syndrome, type 2 diabetes will affect the synthesis and action of osteocalcin and will cause a disruption of the bone-energy metabolism axis, with consequences for the whole organism.

References

1. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. *Biomed Res Int*, 2015, 2015:421746.
2. Oldknow KJ, MacRae VE, Farquharson C. Endocrine role of bone: recent and emerging perspectives beyond osteocalcin. *Journal of Endocrinology*, 2015, 225:R1-R19.
3. Arias CF, Herrero MA, Echeverri LF, Oleaga GE, López JM. Bone remodeling: A tissue-level process emerging from cell-level molecular algorithms. *PLoS One*, 2018,13(9):e0204171.
4. Mathieu Ferron, Julie Lacombe. Regulation of energy metabolism by the skeleton: Osteocalcin and beyond. *Archives of Biochemistry and Biophysics*, 2014, 561:137-146.

5. Fernandes TAP, Gonçalves LML, Brito JAA. Relationships between bone turnover and energy metabolism. *J Diabetes Res*, 2017, 2017:9021314.
6. Confavreux CB. Bone: from a reservoir of minerals to a regulator of energy metabolism. *Kidney Int Suppl*, 2011, 79(121):S14-9.
7. Lin X, Brennan-Speranza TC, Levinger I, Yeap BB. Undercarboxylated Osteocalcin: Experimental and Human Evidence for a Role in Glucose Homeostasis and Muscle Regulation of Insulin Sensitivity. *Nutrients*, 2018, 10(7):pii E847.
8. Han Y, You X, Xing W, Zhang Z, Zou W. Paracrine and endocrine actions of bone-the functions of secretory proteins from osteoblasts, osteocytes, and osteoclasts. *Bone Res*, 2018, 6:16.
9. Liu JM, Rosen CJ, Ducey P, Kousteni S, Karsenty G. Regulation of Glucose Handling by the Skeleton: Insights From Mouse and Human Studies. *Diabetes*, 2016, 65(11):3225-3232.
10. Dumitru N, Carsote M, Cocolos A, Petrova E, Olaru M, Caragheorgheopol A, Dumitrache C, Ghemigian A. Metabolic and bone profile in postmenopausal women with and without type 2 diabetes: a cross-sectional study. *ROM. J. INTERN. MED*, 2018, 0:1-13.
11. Shin JA, Lee JH, Lim SY, et al. Metabolic syndrome as a predictor of type 2 diabetes, and its clinical interpretations and usefulness. *J Diabetes Investig*, 2013, 4(4):334-343.
12. Dumitru N, Cocolos A, Caragheorgheopol A, Dumitrache C, Bratu OG, Neagu TP, Diaconu CC, Ghemigian A. Collagen-The ultrastructural element of the bone matrix. *Revista de Chimie*, 2018, 69(7):1706-1709.
13. Parikh RM, Mohan V. Changing definitions of metabolic syndrome. *Indian J Endocrinol Metab*, 2012, 16(1):7-12.
14. Razny U, Fedak D, Kiec-Wilk B, et al. Carboxylated and undercarboxylated osteocalcin in metabolic complications of human obesity and prediabetes. *Diabetes Metab Res Rev*, 2017, 33:e2862.
15. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, Yano S, Sugimoto T. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes. *J. Clin. Endocrinol. Metab*, 2009, 94:45-49.
16. Saleem U, Mosley TH, Kullo IJ. Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. *Arterioscler Thromb Vasc Biol*, 2010, 30(7):1474-1478.
17. Patti A, Gennari L, Merlotti D, Dotta F, Nuti R. Endocrine actions of osteocalcin. *Int J Endocrinol*, 2013, 2013:846480.
18. Ghemigian A, Radu O, Valea A, Dumitru N, Buruiana A, Carsote M. Hypovitaminosis D and Menopause: still an issue in 2016? *Romanian Medical Journal*, 2016, LXIII(3):209-210.

Corresponding Author: Nicoleta Dumitru, Aviatorilor Ave 34-38, Bucharest, sector 1, postal code 011684, Romania, e-mail: nicoleta3fan@gmail.com