



Relation between osteocalcin and the energy metabolism in obesity

Povezanost osteokalcina i energetskeg metabolizma kod gojaznosti

Stanislava Nikolić*, Nikola Ćurić*, Branislava Ilinčić*, Zoran Stošić*,
Dragana Tomić Naglič†, Damir Benc†

University of Novi Sad, Faculty of Medicine, Clinical Center of Vojvodina,
*Center of Laboratory Medicine, †Clinic for Endocrinology, Diabetes and Metabolic
Disorders, Novi Sad, Serbia

Abstract

Background/Aim. Numerous findings have indicated the potential relation between the osteocalcin, the traditional parameter of bone turnover and the regulation of energy metabolism. The aim of this study was to identify the relationship between osteocalcin and calculated indexes, which evaluate insulin sensitivity, insulin resistance and/or secretory capacity of the pancreas, in non-diabetic, obese subjects. **Methods.** The study included 57 (11 men and 46 women) euglycemic, obese patients (the body mass index – BMI: 41.03 ± 6.61 kg/m²) and 48 healthy individuals, age and sex matched (BMI: 23.15 ± 2.04 kg/m²). Plasma glucose and the insulin levels during the two-hour oral glucose tolerance test (OGTT) were determined in order to calculate the Homeostatic Model Assessment (HOMA) indexes (HOMA-IR, HOMA-B%), EISI (estimated insulin sensitivity index), EFP (estimated first phase) and ESP (estimated second phase). Osteocalcin was measured by using the Electro-chemiluminescence (ECLIA) methodology. **Results.** Statistically lower osteocalcin was found in

the obese subjects (24.72 ± 9.80 vs 33.31 ± 10.89 ng/mL; $p < 0.01$). There was a statistically significant positive correlation between osteocalcin and EISI ($r = 0.340$; $p < 0.01$). The inverse correlations were found between the osteocalcin and HOMA-IR ($r = -0.276$; $p < 0.01$), HOMA-B% ($r = -0.337$; $p < 0.01$), EFP ($r = -0.332$; $p < 0.01$) and ESP ($r = -0.266$; $p < 0.01$). Multiple regression showed that the BMI and osteocalcin have a significant inverse prediction with the EISI and HOMA-IR, but the level of prediction of the BMI was substantially higher. **Conclusion.** The effect of osteocalcin in the glycoregulation is evident, but its contribution is significantly smaller in relation to other obesity associated factors. Therefore, when assessing its position and the role in glycemic control it is always necessary to bear in mind that osteocalcin represents only one of the many contributing factors, some of which exhibit dominant influence than osteocalcin itself.

Key words:

obesity; insulin resistance; osteocalcin; pancreas; body mass index.

Apstrakt

Uvod/Cilj. Brojna dosadašnja saznanja ukazala su na postojanje uloge osteokalcina, tradicionalnog parametra metaboličke aktivnosti kosti, u regulaciji metabolizma ugljenih hidrata. Cilj ove studije bio je da se utvrdi postojanje relacije između osteokalcina i izračunatih parametara procene stepena insulinske osetljivosti/rezistencije i sekretorne sposobnosti pankreasa kod gojaznih, nedijabetičnih ispitanika. **Metode.** Studijom je obuhvaćeno 57 gojaznih osoba (11 muškaraca i 46 žena) (indeks telesne mase – ITM: $41,03 \pm 6,61$ kg/m²) i 48 zdravih, normalno uhranjenih ljudi (ITM: $23,15 \pm 2,04$ kg/m²) koji odgovaraju ispitivanoj grupi, po starosti i polu. Svim ispitanicima izmerena je glukoza i insulin u toku dvočasovnog testa oralnog opterećenja glukozom (OGTT), osteokalcin i izračunate vrednosti *Homeostatic Model Assessment* (HOMA) indeksa (HOMA-IR, HOMA-B%), EISI (indeksa insulinske osetljivosti), (*estimated first phase*) i

EFP (*estimated second phase*) druge faze (ESP) i procena sekretarnog kapaciteta pankreasnih ćelija druge faze (ESP). Koncentracija osteokalcina merena je u serumu, elektrohemiluminescentnom (ECLIA) metodologijom. **Rezultati.** Utvrđene su statistički značajno niže vrednosti osteokalcina u grupi gojaznih (ITM = $24,72 \pm 9,80$ vs $33,31 \pm 10,89$ ng/mL; $p < 0,01$). Linearnom korelacionom analizom dobijen je visok stepen pozitivne povezanosti osteokalcina sa EISI ($r = 0,340$; $p < 0,01$). Inverzna korelacija utvrđena je između osteokalcina i HOMA-IR ($r = -0,276$; $p < 0,01$), HOMA-B% ($r = -0,337$; $p < 0,01$), EFP ($r = -0,332$; $p < 0,01$) i ESP ($r = -0,266$; $p < 0,01$). ITM i osteokalcin imaju značajnu inverznu predikciju sa EISI i HOMA-IR, ali je nivo predikcije ITM bitno viši u odnosu na osteokalcin. **Zaključak.** Uticaj osteokalcina na sistem glikoregulacije je evidentan, ali je njegov udeo u tome bitno manji u odnosu na gojaznost i druge činioce povezane sa gojaznošću. Zbog toga se pri proceni mesta i uloge osteokalcina u glikoregulaciji, uvek mora imati u vidu da je osteokalcin,

kao sistemski medijator, samo jedan od brojnih drugih faktora, pri čemu neki od njih, ispoljavaju dominantnije uticaje od osteokalcina.

Ključne reči:

gojaznost; insulin, rezistencija; osteokalcin; pankreas; telesna masa; indeks.

Introduction

Obesity is defined as a condition of increasing of fat in total body weight and as such represents an important risk factor for the development of many diseases which are based on insulin resistance^{1,2}. Dysfunctional adipose tissue has a high degree of metabolic activity, producing a wide range of humoral mediators that are closely related to the level of bone metabolic activity. Based on these findings, in the early 21st century, a hypothesis was made – obesity affects the metabolic activity of the bones, and that feedback, bone tissue through humoral factors such as osteocalcin may regulate some aspects of glycemic control^{3,4}.

Osteocalcin is one of the most important noncollagenous proteins that participates in the process of bone mineralization. Based on studies on animal models, osteocalcin released in circulation has a prominent role in glycemic control through two main mechanisms: by acting directly on the islet cells of Langerhans, increasing the production and secretion of insulin⁵ and by acting through the indirect mechanisms, primarily through adiponectin^{6,7} on insulin-sensitive cells of the peripheral tissues (muscle and adipose tissue)^{8,9}.

This was followed by clinical studies that examined the relationship between osteocalcin and traditional parameters which evaluate glucose metabolism. For the most part, studies were conducted on subjects with already altered glycemic control, with the values of glycemia over the euglycemic range¹⁰.

In accordance with these facts, this study focuses on the analysis and testing of relations between osteocalcin as a traditional marker of the bone metabolism and the standard laboratory markers of the glucose metabolism.

Methods

This cross-sectional study was conducted in the Department of Endocrine Diagnostics in cooperation with the Outpatient Department of Clinic for Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Vojvodina, during 2015. The study included 57 obese patients (11 men and 46 women) and 48 healthy, normal weight subjects which correspond to the study group by age and gender.

Criteria for exclusion from the study were: disorders of glycemic control (elevated fasting glucose, impaired glucose tolerance and/or diabetes mellitus), endocrinologic diseases, liver diseases which exclude steatohepatitis and include hepatic steatosis), kidney diseases, psychic disorders and the presence of metabolic bone diseases as well as supplementation with vitamin D and/ or calcium preparations.

Anthropometric measurements

To all subjects the body height (BH) and body weight (BW), were measured. The body height was measured by using the Anthropometer according to Martin and is expressed in centimeters (cm); and the BW was determined on the decimal scale in kilograms (kg). The body mass index (BMI) was calculated by using the formula: $BMI (kg/m^2) = BW (kg) / BH^2 (m^2)$ in order to determine the presence of obesity ($BMI > 30 kg/m^2$)¹¹.

Oral glucose tolerance test

After an eight-hour, overnight fasting, standard oral glucose tolerance test (OGTT) was performed to all subjects in order to measure the glucose and insulin plasma levels in basal condition as well as in 120th minute after the oral administration of 75 g of glucose. Blood was taken by venipuncture, using the Vacuette® system. The hemolytic, lipemic and icteric samples were not taken for the analysis. For determination of osteocalcin, the obtained serum samples were frozen at -20 °C and stored until moment of the analysis. All other laboratory parameters were determined directly after obtaining the serum and plasma samples.

Determination of glucose, insulin and glycated hemoglobin A1c

Glucose was measured by the biochemical method (hexokinase) on the Abbott Architect c8000 analyzer by using the commercial kits from the same manufacturer. The reference value for glucose was from 4.1 to 6.1 mmol/L. Insulin was determined by the direct Chemiluminescence technology (CLIA) on the automated system ADVIA Centaur XP. The recommended reference value for insulin in basal conditions is from 3.0 to 25.0 mIU/L. Glycated hemoglobin A1c (HbA1c) was measured by using the immunoturbidimetric method of inhibition of agglutination on microparticles, on the automated system Abbott Architect ci 4100.

Calculating indexes for the evaluation of glycoregulation

In order to evaluate insulin sensitivity, the EISI (estimated insulin sensitivity index) was calculated to all subjects. On that occasion the following formula was used¹²: $EISI = 0.222 - 0.00333 \times BMI - 0.0000779 \times Ins120 - 0.000422 \times age$

were Ins = insulin

As part of the assessment of the secretory capacity of pancreatic beta cells, EFP (estimated first phase) and ESP

(estimated second phase) were calculated by using following formulas ¹²:

$$\text{EFP} = 2.032 + 4.681 \times \text{Ins}0 -$$

$$135.0 \times \text{Gluc}120 + 0.995 \times \text{Ins}120 + 27.99 \times \text{BMI} -$$

$$269.1 \times \text{Gluc}0$$

$$\text{ESP} = 277 + 0.800 \times \text{Ins}0 -$$

$$42.79 \times \text{Gluc}120 + 0.321 \times \text{Ins}120 + 5.338 \times \text{BMI}$$

were Ins = insulin and gluc = glucose

Also, the Homeostatic model assessment (HOMA) indexes (HOMA-IR and HOMA-B%) were determined to all participants by using HOMA 2 calculator. The calculator was downloaded from the official website of the Oxford School of Medicine ¹³, based on the measured values of the blood glucose and insulin levels in basal conditions. A cut-off value for the HOMA-IR is defined as a value less than 2.5 ¹⁴.

Determination of osteocalcin and crosslaps levels

The total osteocalcin (Osteo) was determined on the automated system, Cobas e 411 Roche Diagnostics. The lower limit of detection was 0.5 ng/mL. The osteocalcin values were expressed in ng/mL, and according to the manufacturer, the following reference values were defined – for women; 11–48 ng/mL, and for men: 14–46 ng/mL). The crosslaps was determined on the automated Cobas s 411 system (the reference value of women's reproductive period: 299–573 pg/mL; for men: 300–704 pg/mL).

Statistical analysis

Data are presented using the descriptive statistical methods, continuous variables as mean \pm standard deviation (SD). In order to evaluate the differences between the groups we used the Mann-Whitney U test. The correlations among variables were assessed by the Pearson's correlation

coefficient. The multiple regression analysis was performed to assess the independent association between the BMI, osteocalcin levels and calculated parameters of insulin resistance/sensitivity as well as insulin secretion. A 2-tailed $p < 0.05$ was considered statistically significant. Statistical analysis was performed using the Data Analysis Excel (Microsoft Corp., Redmond, WA) and MedCalc 12.1.4.0 statistical software (MedCalc Software, Mariakerke, Belgium).

Results

The examined group consisted of 57 obese subjects (46 females and 11 males) (BMI : 41.03 ± 6.61 kg/m²). Compared to the control group, the obese subjects differed significantly in all monitored parameters except the blood glucose values in basal, as well as in 120th minute of the standard OGT test ($p = 0.689$; $p = 0.714$). Based on the results, the study group had significantly lower osteocalcin levels ($p < 0.01$) (Table 1).

The linear correlation analysis revealed a significant degree of positive correlation between the osteocalcin levels and the calculated EISI ($r = 0.340$; $p < 0.01$). An inverse correlation was found between osteocalcin and the HOMA-IR ($r = -0.276$; $p < 0.01$), the HOMA-B% ($r = -0.337$; $p < 0.01$), EFP ($r = -0.332$; $p < 0.01$) and the ESP ($r = -0.266$; $p < 0.01$) (Table 2). There was no significant correlation between the osteocalcin and plasma insulin ($r = -0.165$; $p > 0.05$) and glucose levels ($r = -0.007$; $p > 0.05$) after 120 minute of the OGTT as well as with basal glycaemia ($r = 0.137$; $p > 0.05$) while a very low degree of correlation was present with basal insulin ($r = -0.288$; $p < 0.01$). The BMI exhibited a significant correlation with all the examined parameters, but all levels of the correlation were higher than one with osteocalcin. The BMI had a negative correlation only with the EISI ($r = -0.998$; $p < 0.01$) (Table 2).

Table 1

The significance of differences between the examined and control group

Parameters	Obese (n = 57) mean \pm SD	Control (n = 48) mean \pm SD	<i>p</i>
BMI (kg/m ²)	41.03 \pm 6.61	23.15 \pm 2.04	< 0.01
Gluc 0 (mmol/L)	4.94 \pm 1.04	4.75 \pm 0.44	0.238
Gluc120 (mmol/L)	5.44 \pm 1.83	5.02 \pm 1.17	0.172
Ins 0 (mIU/L)	16.86 \pm 9.36	6.15 \pm 3.75	< 0.01
Ins120 (mIU/L)	36.93 \pm 26.11	14.32 \pm 11.44	< 0.01
HbA1c (%)	5.67 \pm 0.79	5.08 \pm 0.38	< 0.01
Osteo (ng/mL)	24.72 \pm 9.81	33.31 \pm 10.89	< 0.01
Csls (pg/mL)	384.40 \pm 190.87	500.90 \pm 207.51	0.003
EISI	0.07 \pm 0.02	0.13 \pm 0.01	< 0.01
HOMA-IR	2.38 \pm 1.29	0.89 \pm 0.54	< 0.01
HOMA-B (%)	186.46 \pm 82.45	97.18 \pm 34.84	< 0.01
EFP	1231.51 \pm 441.81	766.67 \pm 219.89	< 0.01
ESF	289.39 \pm 76.13	195.12 \pm 49.59	< 0.01

Legend: Gluc 0 and gluc 120 – plasma glucose values in 0 and 120 the minute of Oral glucose tolerance test (OGTT); Ins 0 and ins 120 – plasma insulin levels in 0 and 120 minute of OGTT; HbA1c – glycated haemoglobin A1c; Osteo - osteocalcin; Csls – crosslaps; 25OHD – vitamin D; EISI – estimated insulin sensitivity index, calculated according to Stumvoll et al. ¹²; EFP – estimated first phase, calculated according to Stumvoll et al. ¹²; ESP – estimated second phase. calculated according to Stumvoll et al. ¹²; Homeostatic Model Assessment (HOMA)-IR – HOMA index for estimation of the insulin resistance, using HOMA 2 calculator; HOMA-B% – HOMA index for estimation of insulin secretion, using HOMA 2 calculator; *p* – statistical significance.

Table 2

Linear correlation analysis

Parameters	Osteo (ng/mL)		Parameters	BMI (kg/m ²)	
	r	p		r	p
BMI (kg/m ²)	-0.335	< 0.01	Osteo (ng/mL)	-0.335	< 0.01
Gluc 0 (mmol/L)	0.137	> 0.05	Gluc 0 (mmol/L)	0.259	< 0.01
Gluc 120 (mmol/L)	-0.007	> 0.05	Gluc 120 (mmol/L)	0.242	< 0.01
Ins 0 (mU/mL)	-0.288	< 0.01	Ins0 (mU/mL)	0.680	< 0.01
Ins 120 (mU/mL)	-0.165	> 0.05	Ins120 (mU/mL)	0.505	< 0.01
EISI	0.340	< 0.01	EISI	-0.998	< 0.01
HOMA-IR	-0.276	< 0.01	HOMA-IR	0.691	< 0.01
HOMA-B%	-0.337	< 0.01	HOMA-B%	0.558	< 0.01
ESP	-0.266	< 0.01	ESP	0.591	< 0.01
EFP	-0.332	< 0.01	EFP	0.516	< 0.01

Legend: Gluc 0 and gluc 120 – plasma glucose values in 0 and 120 the minute of Oral glucose tolerance test (OGTT); Ins 0 and ins 120 – plasma insulin levels in 0 and 120 minute of OGTT; HbA1c – glyated haemoglobin A1c; Osteo - osteocalcin; Csls – crosslaps; 25OHD – vitamin D; EISI – estimated insulin sensitivity index, calculated according to Stumvoll et al. ¹²; EFP – estimated first phase, calculated according to Stumvoll et al. ¹²; ESP – estimated second phase, calculated according to Stumvoll et al. ¹²; Homeostatic Model Assessment (HOMA)-IR – HOMA index for estimation of the insulin resistance, using HOMA 2 calculator; HOMA-B% – HOMA index for estimation of insulin secretion, using HOMA 2 calculator; p – statistical significance.

Table 3

Multiple regression analysis

Model	Dependent variable	Adjusted R ²	p	Predictors		
				t	p (t)	
A	EISI	0.862	< 0.001	BMI	-11.321	< 0.001
				Osteo	5.207	< 0.001
B	HOMA-IR	0.798	< 0.001	BMI	13.435	< 0.001
				Osteo	-3.109	0.002
C	HOMA-B%	0.835	< 0.001	BMI	13.329	< 0.001
				Osteo	-1.102	0.272
D	EFP	0.874	< 0.001	BMI	14.068	< 0.001
				Osteo	0.655	0.513
E	ESP	0.920	< 0.001	BMI	17.073	< 0.001
				Osteo	1.607	0.361

Legend: Adjusted R² – coefficient of determination that is compliant with the number of independent variables included in the model; p – statistical significance; EISI – estimated insulin sensitivity index; HOMA – homeostatic model assessment; EFP – estimated first phase; ESP – estimated second phase; BMI – body mass index; Osteo – osteocalcin.

The multiple regression analysis was used in order to examine an independent predictive potential of osteocalcin in relation to the individual parameters of glycemic control, in all subjects (Table 3). In the model A (R² = 0.862, p < 0.001), we observed that the serum levels of osteocalcin, positively and independently contribute to the EISI values. Also, the model B (R² = 0.798, p < 0.001) showed that the serum levels of osteocalcin negatively and independently contribute to the HOMA-IR. In addition to a statistically significant predictive impact of osteocalcin on the EISI and HOMA-IR (models A and B), t coefficients indicated that the influence of the BMI to specified indexes was more pronounced than of osteocalcin itself. Osteocalcin, in the models C, D and E did not show a significant predictive potential in relation to the HOMA-B%, EFP and ESP.

Discussion

The examined group consisted of 57 obese patients (obesity grade II and grade III). In comparison to the healthy controls, the group of obese subjects did not differ significantly in the measured

values of the plasma glucose levels during two-hour OGTT. However, the statistically significant elevated HbA1c values may indicate the presence of the modified discrete regime of glycemic control in the group of obese patients.

Our results are in agreement with the known facts that obese subjects compared to normally weighted, ones have decreased insulin sensitivity and/or the increased insulin resistance ¹⁵. According to the obtained results, the examined group had significantly higher levels of insulin in basal conditions and after 120 minutes of the OGT Test, which could be responsible for maintaining the plasma glucose levels in the reference range.

Also, the calculated index that assesses the insulin resistance (HOMA-IR) was significantly higher in the obese, while the indicator of insulin sensitivity (EISI) was significantly lower in the obese compared to the control group. The aforementioned index is widely used in everyday clinical practice and represents a measure of quantifying insulin resistance and insulin sensitivity.

Our analysis showed that obesity had a very high degree of correlation with the parameters of insulin

secretion: the HOMA-B%, EFP and ESP. Due to the fact, obesity is essentially connected to an increased insulin resistance and elevation of insulin secretion reflects the strong compensatory mechanism in order to maintain normoglycemia. The obtained results indicate that in obesity, with clearly altered biological effects of insulin on the effector cells, functioning capacity of the islet cells of Langerhans is completely preserved, and operates in the mode of hypersecretion. Since among the examined group, hiperglycemia or decreased glucose tolerance was not registered, this group of obese patients was characterized by an initial, the mildest form of abnormal glucose regulation.

The obese patients showed statistically lower values of osteocalcin, compared to the control group of healthy subjects. Similar results were obtained by Cifuentes et al.¹⁶ Lucey et al.¹⁷ showed a significantly lower value of the total osteocalcin in the patients with the BMI value equal or lower than to 34.9 kg/m² in comparison to women of normal weight.

Apart from the already mentioned fact that obese people have lower levels of osteocalcin, we found a significant negative correlation between the BMI and osteocalcin levels. It is believed that osteocalcin released from the bone tissue to the circulation is becoming a mediator who stimulates the proliferation of pancreatic beta cells and insulin secretion, and on the other hand increases the insulin sensitivity of peripheral tissue¹⁸⁻²⁰. The mechanism which can explain this relation is an abnormal secretion of leptin in obesity which may decrease bone metabolic activity, primarily through the central, sympathetic regulation of bone cells. It is also known that leptin decreases the expression of adiponectin and its concentration in circulation. In mice, the parenteral administration of osteocalcin increases the expression of adiponectin in white adipose tissue due to the improvement of insulin sensitivity, reduction of triglycerides in the liver and muscles as well as the inhibition of gluconeogenesis²¹⁻²³.

Osteocalcin shows a highly significant correlation with the EISI and HOMA-IR which make it one of indisputable regulators of the biological effects of insulin on the cell level. In respect of osteocalcin, obesity exerts the opposite effects in terms of the increased insulin resistance and decreased insulin sensitivity. Also, it can be seen that compared to osteocalcin, obesity has a significantly higher level of association with the insulin resistance and/or sensitivity. These results indicate that obesity is predominantly related to the increased insulin resistance and reduced insulin sensitivity, while the preferred level of osteocalcin connection to the manifestation of the biological insulin effects is substantially lower. The current level of the increased insulin resistance and reduced sensitivity in the group of the obese, is predominantly caused by the adverse effects of obesity in opposite to the protective effects of osteocalcin.

At first glance, the correlation results a great surprise are, which showed significant inverse relationship between osteocalcin and the already mentioned indicators of insulin secretion (HOMA-B%, EFP, ESP). These results are in stark contrast to the so far known facts suggesting that osteocalcin stimulates beta cells of Langerhans and insulin secretion²⁴. This mechanism is mediated by the action of osteocalcin *via* the Gprc6a receptors by stimulating the proliferation of beta cells. Also, it is known that through the same receptor at the level of the gastrointestinal tract, osteocalcin stimulates the production of GLP-1 (glucagon like peptide-1) and in that way participate in the preservation of insulin secretion^{7, 25}. The explanation for the obtained relation can be found in the fact that the study was conducted among obese people with the minimal disturbances of the glycemic control and subsequent insulin hypersecretion. Logical processing of the data shows: the greater obesity, the lower osteocalcin, and higher insulin secretion. On the basis of this fact, it can be concluded that between osteocalcin and insulin secretion there is an inverse correlation.

In order to comprehensively examine the influence of different factors on the insulin secretion in obese subjects, the multiple linear regression analysis was performed. It is necessary to bear in mind that the factor of obesity is complex and comprises a plurality of factors that directly or indirectly influence both insulin secretion and insulin resistance/sensitivity. This statistical method showed that osteocalcin has no significant prediction of insulin secretion (the HOMA-B%, EFP, ESP), opposite to the complex factor of obesity (BMI). Therefore, it could be concluded that the chosen model of this research, although conducted on the small sample size, is not suitable for studying the influence of osteocalcin on insulin secretion.

Conclusion

The obtained significantly lower concentrations of osteocalcin in the obese subjects, compared with those of normal weight, are the consequences of the altered energy metabolism in obesity.

The inverse relationship between osteocalcin and obesity indicate an actual connection between the bone metabolism and glycemic control in the complex system of the energy metabolism.

The impact of osteocalcin on glycemic control is evident, but its share is substantially lower in relation to obesity and other factors associated with obesity. Therefore, when assessing the place and role of osteocalcin in the glycemic control, there should always be taken into account that osteocalcin, as a systemic mediator, is only one of many other, less influential factors.

R E F E R E N C E S

1. *Lebovitz HE*. Insulin resistance: Definition and consequences. *Exp Clin Endocrinol Diabetes* 2001; 109 Suppl 2: S135-48.
2. *Ionescu-Tirgoviste C, Ioacara S, Guja C, Sabau S, Lichiardopol R, Mihai A*, et al. A pathophysiological approach to metabolic syndrome using factor analysis in an adult Romanian population. *Arch Physiol Biochem* 2006; 112(3): 182-8.
3. *Fukumoto AS, Martin J*. Bone as an endocrine organ. *Trend Endocrinol Metab* 2009; 20(5): 230-6.

4. Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. *Trend Endocrinol Metab* 2008; 19(5): 161–6.
5. Lee NK, Sowa H, Hinoi E, Ferron M, Abn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007; 130(3): 456–69.
6. Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. *Cell Metab* 2006; 4(5): 341–8.
7. Crivjovic G, Micić G, Kendereški A, Zoric S, Polovina S, Šumarac DM, et al. Glucose and bone metabolism – potential interactions. *Acta Clin* 2014; 14(3): 68–72. (Serbian)
8. Motyl KJ, McCabe LR, Schwartz AV. Bone and glucose metabolism: A two-way street. *Arch Biochem Biophys* 2010; 503(1): 2–10.
9. Wiecek A, Adamczak M, Chudek J. Adiponectin: an adipokine with unique metabolic properties. *Nephrol Dial Transplant* 2007; 22(4): 981–8.
10. Aoki A, Muneyuki T, Yoshida M, Munakata H, Ishikawa SE, Sugawara H, et al. Circulating osteocalcin is increased in early-stage diabetes. *Diabetes Res Clin Pract* 2011; 92(2): 181–6.
11. Caballero B. The global epidemic of obesity: an overview. *Epidemiol Rev* 2007; 29: 1–5.
12. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van HT, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 2000; 23(3): 295–301.
13. HOMA calculator software. Available from: <https://www.dtu.ox.ac.uk/homacalculator/>
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7): 412–9.
15. Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity. *Curr Opin Endocrinol Diabetes Obes* 2012; 19(2): 81–7.
16. Cifuentes M, Johnson MA, Lewis RD, Heymsfield SB, Chondbury HA, Modlesky CM, et al. Bone turnover and body weight relationships differ in normal weight compared to with heavier postmenopausal women. *Osteoporosis Int* 2003; 14: 116–22.
17. Lucey AJ, Pashos GK, Thorsdottir I, Martinez JA, Cashman KD, Kiel M. Young overweight and obese women with lower circulating osteocalcin concentrations exhibit higher insulin resistance and concentrations of c-reactive protein. *Nutr Res* 2013; 33(1): 67–75.
18. Matthei S, Stumvoll M, Kellerer M, Häring HU. Pathophysiology and pharmacological treatment of insulin resistance. *Endocr Rev* 2000; 21(6): 585–618.
19. Sarkar PD, Choudhury AB. Relationships between serum osteocalcin levels versus blood glucose, insulin resistance and markers of systemic inflammation in central Indian type 2 diabetic patients. *Eur Rev Med Pharmacol Sci* 2013; 17(12): 1631–5.
20. Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmö, Sweden. *Diabet Med* 2000; 17(4): 299–307.
21. 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): 1474–8.
22. Yamamuchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; 423(6941): 762–9.
23. Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates bone cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proc Natl Acad Sci USA* 2008; 105(13): 5266–70.
24. Kanazawa I, Yamaguchi T, Tada Y, Yamauchi M, Yano S, Sugimoto T. Serum osteocalcin level is positively associated with insulin sensitivity and secretion in patients with type 2 diabetes. *Bone* 2011; 48(4): 720–5.
25. Kanazawa I. Osteocalcin as a hormone regulating glucose metabolism. *World J Diabet* 2015; 6(18): 1345–54.

Received on March 28, 2017.

Revised on April 21, 2017.

Accepted on May 22, 2017.

Online First May, 2017.