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Relation of osteocalcin with insulin resistance and histopathological changes of non alcoholic fatty liver disease

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ABSTRACT

Background. Osteocalcin is a hormone with a complex cross-talk between adipose tissue and the skeleton. The aim of the present study was to explore the relation of osteocalcin with histopathological changes of NALFD patients. **Subjects.** A population of 69 NAFLD patients was analyzed. A liver biopsy was realized. Weight, fat mass, body mass index, basal glucose, insulin, insulin resistance (HOMA), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and osteocalcin levels were measured. **Results.** Patients were divided in two groups by median osteocalcin value (11.34 ng/mL), group I (patients with the low values) and group II (patients with the high values). Only liver fibrosis frequencies were different between groups (group I: 22.9% vs group II: 9.4%; $p < 0.05$). Patients in group I had higher levels of glucose (115.6 ± 28.1 mg/dL *vs.* 103.7 ± 24.3 mg/dL; $p < 0.04$), HOMA (4.6 ± 3.1 units *vs.* 3.6 ± 1.8 units; $p < 0.04$), weight (102.9 ± 32.4 kg *vs.* 85.9 ± 16.8 kg; $p = 0.002$) and body mass index (38.3 ± 11.4 kg/m^2 *vs.* 30.1 ± 5.7 kg/m^2; $p = 0.001$)) than patients in group II. Osteocalcin was inverse correlated with glucose ($r = -0.4$; $p = 0.002$) and HOMA ($r = -0.3$:$p = 0.01$). **Conclusion.** Osteocalcin is associated with liver fibrosis. However, this association disappeared in a multivariate analysis, and HOMA remained as an independent factor.

Key words. Adipose Tissue. Non Alcoholic Fatty Liver Disease. Obesity. Osteocalcin.

INTRODUCTION

Epidemiologic evidence of the rising tide of obesity and associated pathologies has led, in this century, to a dramatic increase of research on the role of adipose tissue as an active participant in controlling the body's physiologic and pathologic processes.[1] The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, fat liver deposits and bone formation.

Osteocalcin has been viewed as a constituent of the bone extracellular matrix. Osteocalcin contains three glutamic acid residues that are gamma carboxylated (gla residues),[2] hence the alternative name of osteocalcin, bone gla protein.[3] Because this postraslational modificaction confers high affinity for minerals, it was thus assumed that osteocalcin is involved in bone extracellular matrix mineralization.[4] Also, osteocalcin shows some features of hormone. For instance, it is encoded by a cell-specific gene and it is present in blood. Homozygous osteocalcin-gene deficient mice have low insulin levels, high blood glucose levels, and decreased insulin secretion and insulin sensitivity.

Patients with chronic liver disease are at increased risk of develop metabolic bone disease.[6] The etiology of the osteopathy of patients with chronic liver disease has not been clarified untile now; among other causes, decreased serum osteocalcin levels has been related with this entity.[7] Nonalcoholic fatty liver disease (NAFLD) is a common liver disease characterized by elevated serum aminotransferase levels, hepatomegaly and accumulation of fat in liver accompanied by inflammation and necrosis resembling alcoholic hepatitis in the absence of heavy alcohol consumption.[8] It is important to discern what factors in the host metabolic milieu modulate

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the development of NAFLD. Perhaps, the role of osteocalcin on insulin actions could influence on histopathological changes of NAFLD.

To the best of our knowledge, a well-defined group of patients with NAFLD has not been examined so far for influence of osteocalcin levels in liver changes. Accordingly, the aim of the present study was to explore the relation of osteocalcin with underlying histopathological characteristics of NALFD patients.

SUBJECTS AND METHODS

Subjects and procedure

A population of 69 NAFLD patients was analyzed in a cross sectional study in a hospital-based setting. Patients were recruited in a consecutive prospective way in the Digestive Departament. The exclusion criteria were hepatitis B, C, cytomegalovirus, Epstein Barr infections, monogram-specific auto antibodies, alcohol consumption, diabetes mellitus, intolerance fasting glucose, medication (and diabetic drugs, blood-pressure lowering medication and statins) and hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency). Diabetes mellitus and intolerance fasting glucose were has been excluded with basal glucose after 8 hours of fasting state.[9] The study was approved by an institutional ethics committee.

A liver biopsy was realized. Weight, basal glucose, insulin, insulin resistance (HOMA), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and osteocalcin blood levels were measured. A bioimpedance was performed.

Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL cholesterol was calculated using Friedewald formula. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by enzymatic colorimetric (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values.[10]

Total osteocalcin was analyzed in duplicated using a commercially available ELISA kit (Immunodiagnostics System Ltd (IDS Ltd), Boldon, UK). Assay sensitivity was 0.5 ng/mL and interassay and intraassay coefficients of variation were less than 5.1 and less than 2.2, respectively.

Anthropometric measurements

Body weight was measured to an accuracy of 0.1 kg and body mass index computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition[11] (Biodynamic Model 310e, Seattle, WA, USA).

Liver biopsy

Liver tissues were stained with hematoxylin-eosin, reticulin, and Gomori trichrome stains and scored by an experienced hepathologist. All cases showed macrovesicular steatosis affecting at least 5% of hepatocytes and were classified as steatosis. In addition to steatosis, the minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus. All cases were scored using the method of Brunt.[12] Steatosis was graded as follows: grade 1 (> 5% and < 33% of hepatocytes affected); grade 2 (33-66%); or grade 3 (> 66% of hepatocytes affected). Grades 2 and 3 were combined for statistical analysis (high grade) and grade 1 (low grade). Fibrosis was assessed with the Masson trichome stain.[13] Other histological features evaluated in haemtaoxylin-eosin sections included lobulillar inflammation and portal inflammation.

Statistical analysis

The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, unpaired Student's-t test. Non-parametric variables were analyzed with the U Mann Whitney. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. Correlation analysis was realized with Pearson test. Additionally, logistic regressions

with stepwise variable selection were used to test for significant relations in histopathological lesions (steatosis, fibrosis, lobulillar and perinusoidal inflammation) with adjustment for possible confounders. A p-value under 0.05 was considered statistically significant. SPSS 15.0 software (IL, USA) was used.

RESULTS

Sixty-nine patients gave signed informed consent and were enrolled in the study (approved by Ethical Committee of HCU). The mean age was 43.2 ± 12.2 years, the mean BMI was 33.8 ± 11.2 with 44 males (64.3%) and 25 females (35.7%).

Table 1 shows differences between high grade of steatosis and low grade of steatosis. Patients with high grade of steatosis have higher levels of weight, body mass index, insulin and HOMA levels than low grade steatosis.

Table 2 shows differences between patients with lobular inflammation *vs.* no lobular inflammation. Patients with this type of inflammation have higher levels of body mass index, weight and fat mass than patients without inflammation.

Table 3, shows differences between patients with portal inflammation *vs.* no portal inflammation. Patients with this type of inflammation have higher levels of body mass index, weight, fat mass, in-

Table 1. Clinical and epidemiological characteristics (low grade *vs.* high grade of steatosis).

Characteristics (Mean ± Sd)	LOW GRADE n = 30	HIGH GRADE n = 39	p
Age (years)	45.7 ± 11.1	41.4 ± 12.9	0.14
Sex (male/female)	9/21	16/23	0.48
BMI (kg/m^2)	31.0 ± 7.3	36.8 ± 11.4*	0.02
Weight (kg)	85.3 ± 18.3	101.7 ± 11.3*	0.02
Fat mass (kg)	24.2 ± 9.1	26.2 ± 8.1	0.52
Total cholesterol (mg/dL)	203.3 ± 56.1	184.1 ± 44.2	0.14
LDL-cholesterol (mg/dL)	134.1 ± 50.7	112.8 ± 35.8	0.18
HDL-cholesterol (mg/dL)	52.9 ± 12.1	52.1 ± 28.2	0.89
Glucose (mg/dL)	106.2 ± 26.1	113.1 ± 7.3	0.31
HOMA-IR	2.7 ± 1.5	5.1 ± 2.8*	0.001
Insulin (mUI/L)	10.3 ± 3.6	18.7 ± 11.3*	0.001
Osteocalcin (ng/mL)	13.9 ± 8.1	12.4 ± 5.9	0.37

BMI: Body mass index. **LDL:** Low density lipoprotein. **HDL:** High density lipoprotein. **HOMA-IR:** Homeostatic model assessment (glucose (mmol/L*insulin uU/mL)/22.5). T-Student test used. Insulin and HOMA-IR have non-parametric distribution (U-Mann test used). (*) $p < 0.05$.

Table 2. Clinical and epidemiological characteristics lobulillar inflammation (no inflammation *vs.* lobulillar inflammation).

Characteristics (Mean ± Sd)	NO INFLAMMATION n = 28	LOBULAR INFLAMMATION n = 41	p
Age (years)	42.7 ± 12.4	43.6 ± 12.2	0.48
Sex (male/female)	22/6	22/19*	0.04
BMI (kg/m^2)	31.4 ± 7.2	36.5 ± 11.4*	0.04
Weight (kg)	88.3 ± 15.3	99.2 ± 31.3*	0.02
Fat mass (kg)	21.7 ± 9.1	26.8 ± 7.8*	0.05
Total cholesterol (mg/dL)	204.1 ± 60.2	192.2 ± 45.9	0.12
LDL-cholesterol (mg/dL)	126.1 ± 54.1	119.6 ± 37.4	0.61
HDL-cholesterol (mg/dL)	53.3 ± 11.9	52.0 ± 27.1	0.81
Glucose (mg/dL)	106.6 ± 12.1	112.5 ± 27.3	0.38
HOMA-IR	3.4 ± 2.6	4.6 ± 2.5	0.68
Insulin (mUI/L)	13.2 ± 12.1	16.2 ± 7.7	0.38
Osteocalcin (ng/mL)	14.5 ± 8.8	12.1 ± 5.1	0.15

BMI: Body mass index. **LDL:** Low density lipoprotein. **HDL:** High density lipoprotein. **HOMA-IR:** Homeostatic model assessment (glucose (mmol/L*insulin uU/mL)/22.5). T-Student test used. Insulin and HOMA-IR have non-parametric distribution (U-Mann test used). (*) $p < 0.05$.

sulin and HOMA than patients without inflammation.

Table 4, shows differences between patients with fibrosis vs. no fibrosis. Patients with fibrosis have higher levels of insulin, HOMA BMI, and fat mass than patients without fibrosis. However, patients with fibrosis have lower levels of osteocalcin.

Patients were divided in two groups by median osteocalcin value (11.34 ng/mL), group I (patients with the low values) and group II (patients with the

Table 3. Clinical and epidemiological characteristics portal inflammation (no inflammation *vs.* portal inflammation).

Characteristics (Mean ±-Sd)	NO INFLAMMATION n = 55	PERINUSOIDAL INFLAMMATION n = 14	p
Age (years)	44.1 ± 12.5	39.6 ± 12.6	0.22
Sex (male/female)	38/17	6/8*	0.03
Weight (kg)	90.5 ± 18.7	110.1 ± 43.1*	0.01
BMI (kg/m^2)	32.7 ± 8.5	40.2 ± 13.6*	0.02
Fat mass (kg)	24.5 ± 8.3	28.4 ± 8.7*	0.02
Total cholesterol (mg/dL)	199.2 ± 55.2	189.2 ± 40.1	0.78
LDL-cholesterol (mg/dL)	123.8 ± 46.2	115.3 ± 39.1	0.78
HDL-cholesterol (mg/dL)	51.2 ± 12.3	57.3 ± 42.9	0.40
Glucose (mg/dL)	108.1 ± 27.1	117.7 ± 23.2	0.24
HOMA-IR	3.6 ± 2.4	5.7 ± 2.6*	0.008
Insulin (mUI/L)	13.7 ± 9.8	19.6 ± 8.7*	0.04
Osteocalcin (ng/mL)	13.5 ± 7.5	11.2 ± 3.2	0.26

BMI: Body mass index. **LDL:** Low density lipoprotein. **HDL:** High density lipoprotein. **HOMA-IR:** Homeostatic model assessment (glucose (mmol/L*insulin uU/mL)/22.5). T-Student test used. Insulin and HOMA-IR have non-parametric distribution (U-Mann test used). (*) $p < 0.05$.

Table 4. Clinical and epidemiological characteristics liver fibrosis (no fibrosis *vs.* fibrosis).

Charactersitics (Mean ± Sd)	No fibrosis n = 58	Fibrosis n = 11	p
Age (years)	42.7 ± 11.9	46.0 ± 14.2	0.42
Sex (male/female)	40/18	4/7*	0.04
BMI (kg/m^2)	32.4 ± 7.6	43.8 ± 15.6*	0.001
Fat mass (kg)	23.9 ± 8.1	30.6 ± 8.2*	0.02
Total cholesterol (mg/dL)	197.9 ± 49.3	192.5 ± 68.4	0.76
LDL-cholesterol (mg/dL)	120.4 ± 36.8	132.8 ± 79.3	0.47
HDL-cholesterol (mg/dL)	50.5 ± 13.4	64.2 ± 46.7	0.08
Glucose (mg/dL)	109.6 ± 27.8	112.2 ± 27.1	0.77
HOMA-IR	3.7 ± 2.4	5.9 ± 2.9*	0.02
Insulin (mUI/L)	13.9 ± 9.6	20.6 ± 9.7*	0.04
Osteocalcin (ng/mL)	13.7 ± 7.2	9.6 ± 3.7*	0.01

BMI: Body mass index. **LDL:** Low density lipoprotein. **HDL:** High density lipoprotein. **HOMA-IR:** Homeostatic model assessment (glucose (mmol/L*insulin uU/mL)/22.5). T-Student test used. Insulin and HOMA-IR have non-parametric distribution (U-Mann test used). (*) $p < 0.05$.

Tabla 5. Liver histopathological changes by median osteocalcin.

Frequencies	Low osteocalcin n = 35	High osteocalcin n = 34	p
High grade of esteatosis	54.3%	60.6%	0.53
Lobulillar inflammation	62.9%	56.3%	0.58
Portal inflammation	20.0%	21.9%	0.85
Fibrosis	22.9%	9.4%*	0.04

High frequency of portal inflammation in high visfatin group, Other pathological changes without differences. Chi square test used (*) $p < 0.05$.

high values). Table 5 shows the statistical differences between both groups in liver biopsy characteristics. Only liver fibrosis frequencies were different between groups (low osteocalcin group: 22.9% *vs.* high osteocalcin group: 9.4%; $p < 0.05$). Patients in group I had higher levels of glucose (115.6 ± 28.1 mg/dL *vs.* 103.7 ± 24.3 mg/dL; $p < 0.04$), HOMA (4.6 ± 3.1 units *vs.* 3.6 ± 1.8 units; $p < 0.04$), weight (102.9 ± 32.4 kg *vs.* 85.9 ± 16.8 kg; $p = 0.002$) and body mass index (38.3 ± 11.4 kg/m^2 *vs.* 30.1 ± 5.7 kg/m^2; $p = 0.001$)) than patients in group II. Osteocalcin was inverse correlated with glucose ($r = -0.4$; $p = 0.002$) and HOMA ($r = -0.3$;$p = 0.01$).

In the logistic regression with age-, sex-, BMI, fat mass- and insulin- adjusted portal inflammation, high grade of steatosis, fibrosis and lobular inflammation as dependent variables, osteocalcin concentrations are no related with histological changes. HOMA is related with portal inflammation 1.31(CI95%:1.05-1.64), with high grade of steatosis 1.95(CI95%:1.29-2.91) and with fibrosis 1.29(CI95%:1.02-1.63), secondaries to an increase of one unit of HOMA.

DISCUSSION

The present study demonstrates that osteocalcin is associated with liver fibrosis. However, this association disappeared in a logistic regression model, and only HOMA remained in the model.

The relationship between liver histopathological changes and biochemical parameters are a unclear topic area in NAFLD patients. In obese patients, the primary abnormality may be genetically induced insulin resistance, with a secondary increase of serum triglyceride levels due to enhance of peripheral lipolysis. The resulting hepatic supply of fatty acids and insulin may increase triglyceride deposition in the liver.[14-15] In our study steatosis, portal inflammation and fibrosis were related with HOMA, this data confirmed previous hypothesis.

The novel finding of our study is the relation of osteocalcin with liver fibrosis, perhaps as a surrogate marker of insulin resistance. An increasing number of studies have indicated the presence of a complex cross-talk between adipose tissue and the skeleton. Surprinsingly, Lee, *et al.*[5] showed that osteocalcin affects adiposity and glucose homeostasis in mice, suggesting that the skeleton influences on energy metabolism. Osteocalcin-deficient mice displayed obesity, hyperglycemia and insulin resistance. When ostoecalcin was administered to these mice, blood glucose decreased.[5] Recently, Kindblom, *et al.*[16] showed that osteocalcin was an independent negative predictor of plasma glucose in elderly humans. These findings suggest that previous described endocrine function of the osteoblats-derived osteocalcin on glucose homeostasis in mice also might exist in humans and could influence in some entities of metabolic syndrome sush as NAFLD. We herein show that plasma levels of osteocalcin were clearly negatively associated with glucose, HOMA and liver fibrosis in patients with NAFLD. However, adjusted logistic regression models showed that osteocalcin was not an independent negative predictor of liver fibrosis. The effect on liver fibrosis is mediated by insulin resistance.

In the literature, levels of osteocalcin were lower in the diabetic subjects than in the nondiabetic subjects.[17] Nevertheless, osteocalcin had a clear negative correlation with weight and plasma glucose both before and after exclusion of the diabetic patients,[16] indicating that the association between osteocalcin and glucose homeostasis is not caused by diabetes mellitus. However, other possible explanation of the inverse association between osteocalcin and glucose homeostasis is that obesity is associatd with low bone turnover, as Khosla, *et al.* described.[18]

Secondly, osteocalcin may be an epiphenomenon of an inflammatory state of obese patients with NAFLD, without a direct effect on liver dammage. For example, Pittas, *et al.*[19] have demonstrated that serum osteocalcin was inversely associated with glucose, HOMA, BMI and inflammatory markers such as C reactive protein and interleukine-6.

The main limitation of our study is that it is a cross-sectional study and, therefore, one should be cautious with mechanistic interpretations of our finding. However, to the best of our knowledge, a well-defined group of patients with NAFLD has not been examined so far for influence of osteocalcin levels in liver changes. Only, Szalay, *et al.*[20] have demonstrated a decreased serum osteocalcin levels in non-alcoholic and alcoholic chronic liver disease, without a histological evaluation of the liver. In this context, our preliminary datas could be important in this "metabolic puzzle".

The reasons for these unclear results in the literature are unclear but may have been caused by the following factors. First, criteria for recruitment were different in the various studies, thus differences in confounding factors such as age, presence of diabetes mellitus, presence of NAFLD, degree of obesity may affect results. Second, perhaps ethnic heterogeneity may affect osteocalcin expression, too. Finally, genetic background with other different ge-

netic single nucleotide polymorphisms in the osteocalcin way could influence osteocalcin interaction with metabolic parameters.

Our study design cannot explain causality, but the main strength is the liver histology, using biopsy. Further interventional studies to increase osteocalcin levels[5] are needed to explore histopathological improvements in liver biopsy. Dose-response experiments in animals, confirmed the dose effect of osteocalcin on insulin expression by cultured beta cells plateaued at high concentration of osteocalcin.[21] Moreover, osteocalcin levels could predict the presence of the liver fibrosis, this molecule could involve a non invasive technical to determine this pathological change with HOMA, too. Recently, Saleem, *et al.*[22] have demonstrated that serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of MetSyn, suggesting a novel cross-talk between bone and adipose tissue. This association raises interesting new prospects for future research in this area of work.

In conclusion, osteocalcin is associated with liver fibrosis. This univariate analysis would be important if in further studies a clinical consequence either in liver disease or perhaps in bone metabolism could be demonstrated.

REFERENCES

1. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin and resistin. *Clin Chemistry* 2004; 50: 1511-25.
2. Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. *Trends in Endocrinology and Metabolism* 2009; 19: 161-6.
3. Hauschka PV. Osteocalcin and matrix Gla protein :vitamin K dependent proteins in bone. *Physiol Rev* 1989; 69: 990-1047.
4. Price PA. Gla-containing proteins of bone. *Connect Tissue Res* 1989; 21: 51-7.
5. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, COnfavreux C, DAcquin R, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007; 130: 456-69.
6. George J, Ganesh HK, Acharya S, Bandgar T, Shivane V, Karvat A, et al. Bone mineral density and disorders of mineral metabolis in chronic liver disease. *W J Gastroenterology* 2009; 15: 3516-22.
7. Pietschmann P, Resch H, Muller Ch, Woloszczuk W, Willnonseder R. Decreased serum ostoecalcin levels in patients with liver cirrhosis. *Bone and mineral* 1990; 8: 103-8.
8. Ludwig J, Viggiano TR, McGill DB, Oh BJ: Nonalcoholic steatohepatitis: Mayo Clinic experiencies with a hitherto unnamed disease. *Mayo Clinic Proc* 1980; 55: 434-8.
9. Standards of medical carein Diabetes 2007. *Diabetes Care* 2008; 30: s4-s41.
10. Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher Df. Homesotasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412-14.
11. Pichard C, Slosman D, Hirschel B, Kyle U. Bioimpedance analysis in patients: an improved method for nutritional follow up. *Clin Res* 1993; 41: 53ª.
12. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis* 2001; 21: 3-16.
13. Bondini S, Kleiner DE, Goodman ZD, Gramlich T, Younossi ZM. Pathologic assesment of non-alcoholic fatty liver disease. *Clin Liver Dis* 2007; 11: 17-23.
14. Venturi C, Zoppini G, Zamboni C, Muggeo M: Insulin sensivity and hepatic steatosis in obese subjects with normal glucose tolerance. *Nutr Metab Cardiovsc Dis* 2004: 14: 200-4.
15. Browning JD, Horton JD: Molecular mediators of hepatic steatosis and liver injury. *The J of Clin Invest* 2004: 114: 147-52.
16. Kindblom JM, Ohlsson C, Ljunggren O, Karlsson M, Tivesten A, Smith U, Mellstrom D. Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. *J of Bone and Mineral Research* 2009; 24: 785-91.
17. Gerdhem P, Isaksson A, Akesson K, Obrant KJ. Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus. *Osteoporosis Int* 2005; 16: 1506-12.
18. Khosla S, Atkinson EJ, Riggs BL, Melton LJ. Relationship between body composition and bone mass in women. *J Bone Miner Res* 1996; 11: 857-63.
19. Pittas A, Harris S, ELiades M, Stark P, Dawson B. Association between serum osteocalcin and markers of metabolic phenotype. *J Clin Endocrin Metab* 2008 as doi:10.1210/jc.2008-1422.
20. Szalay F, Lakatos P, Németh J, Abonyi M, Büki B, Tarján G, Holló I. Decreased serum osteocalcin level in non-alcoholic and alcoholic chronic liver diseases. *Orv Hetil* 1991; 132(24): 1301-5.
21. Feron M, Hinoi E, KArsenty G, Ducy P. Osteocalcin differentally regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild type mice. *Proc Natl Acad Sci USA* 2008; 105: 5266-70.
22. Saleem U, Mosley TH Jr, 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.