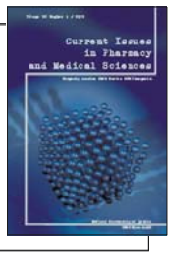
2012 © Curr. Issues Pharm. Med. Sci. Vol. 25, No. 4, Pages 367-372

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy



Selected markers of bone turnover in type 2 diabetic patients

ARLETA MALECHA-JĘDRASZEK[1], AGATA BURSKA[3], HELENA DONICA[1], BEATA MATUSZEK[2], MACIEJ KORPYSZ[1], BEATA WOJTYSIAK-DUMA[1], ANDRZEJ NOWAKOWSKI[2]

[1] Department of Biochemistry Diagnostics, Medical University of Lublin, Poland
[2] Department of Endocrinology, Medical University of Lublin, Poland
[3] Institute of Molecular Medicine, University of Leeds, UK

ABSTRACT

The mechanisms of bone alterations in diabetes mellitus type 2 (DM2) are complex and still poorly understood. It depends on the number of protective factors on bone health, such as hyperinsulinemia, obesity, estrogens as well as factors accelerating bone resorption (e.g. hyperglycemia). The aim of this study was to evaluate the concentrations of CTx (C-terminal cross-linked telopeptide of the alpha chain of type I collagen) and OCN (osteocalcin) in DM2 patients and to look for correlations between these markers and selected clinical data as well as biochemical parameters evaluated in routine diagnostics and monitoring of diabetes (glucose, lipid profile, urea, creatinine, parathyroid hormone, HbA1c, ALT, AST and alkaline phosphatase). The study was conducted in 45 patients with type 2 diabetes aged 60.7±10.3 years. The control group consisted of 25 healthy subjects aged 57.6±11.9 years. In DM2 patients the concentrations of OCN (14.5±8.8 μg/ml; $p<0.001$), CTx (0.3±0.2 μg/ml; $p<0.05$) were significantly lower than in healthy subjects (28.1±11.9 μg/ml, 0.5±0.2 μg/ml for OCN and CTx, respectively). Significantly higher levels of OCN ($p<0.05$) and CTx ($p<0.01$) were found in women than in men within the study group. In DM2 patients OCN concentration was positive correlated with CTx ($r=0.721$, $p=0.000$), PTH ($r=0.426$, $p=0.003$) and negative with TG ($r=-0.349$, $p=0.019$). Furthermore, CTx was directly correlated with ALP ($r=0.396$, $p=0.009$) and PTH ($r=0.413$, $p=0.005$) as well as inversely with TG ($r=-0.302$, $p=0.044$) and HbA1c ($r=-0.349$, $p=0.019$). In conclusion, the observed lower levels of OCN and CTx and their positive inter-correlation may suggest overall slowed down bone metabolism with reduced bone formation and bone resorption in patients with DM2.

Keywords: type 2 diabetes, bone turnover, bone markers, osteocalcin, CTx

INTRODUCTION

Diabetes mellitus type 2 (DM2) is widely recognized serious health problem among developed and developing countries and it appears to be related to the changes from traditional patterns of life towards Western lifestyle. It is recognized as civilization disease, which occur in epidemic proportions in modern societies. Two important elements contribute significantly to the pathogenesis of DM2; tissue insulin resistance and pancreatic β cell dysfunction. It is believed that in many cases DM2 is diagnosed too late; when chronic complications are already present [5,19].

Vast majority of literature show that diabetes and related metabolic disturbances can lead to significant changes in bone metabolism [1,3,4,20,14]. Regardless the differences in bone mineral density in patients with DM2 abnormalities in bone formation can be seen. Decreased quality of the bone as well as impaired micro- and macroarchitecture together with susceptibility to falls resulting from the presence of chronic complications leads to a greater incidence of fractures in DM2 patients [3,14,10,24].

The mechanisms of bone alterations in DM2 are complex and still poorly understood. It depends on the number of protective factors on bone health, such as hyperinsulinemia, obesity, estrogens as well as factors accelerating bone resorption (e.g. hyperglycemia) [7,21, 24]. Therefore evaluation of bone metabolism in patients with DM2 undertaken in this study seems to be fully justified. In the present study we focused on the following bone markers CTx (C-terminal cross-linked telopeptide of the alpha chain of type I collagen) and OCN (osteocalcin). CTx levels reflect type I collagen degradation and indirectly represents bone resorption by the osteoclasts and loss of bone mass. OCN however is known as a highly specific marker of bone turnover rate and the activity of the osteoblasts [1,9,20,24]. The aim of this study was to

Corresponding author
* Department of Biochemistry Diagnostics, Medical University of Lublin, 11 Staszica St., 20-081 Lublin, Poland
e-mail: a.malecha@wp.pl

evaluate the concentrations of CTx and OCN in obese DM2 patients and to compare it with levels obtained in matched healthy population with normal body mass. The second aim was to look for correlations between these markers and selected clinical data as well as other biochemical parameters evaluated in routine diagnostics and monitoring of diabetes.

MATERIALS AND METHODS

Characteristics of the study and control groups. The study was conducted in 45 patients aged 60.7±10.3 years, who were diagnosed with type 2 diabetes. The study group consisted of 22 women aged 63.5±11.7 years and 23 men aged 58.0±8.1 years. All patients were treated at the Department of Endocrinology of the Medical University of Lublin. The average time from the diabetes onset was 9.2±7.3 years.

All patients underwent clinical examination. The prevalence of chronic complications of the disease were clinically evaluated. Hypertension was present in 87% cases, ischemic heart disease in 33% and myocardial infarction in 11% cases. Average BMI (body mass index) was 33.2±4.9 kg/m^2. According to WHO (World Health Organization) criteria [26] 13% of patients were classified as overweight (BMI 25.0-29.9 kg/m^2), 78% as obese (BMI 30.0-39.9 kg/m^2) and 9% as morbidly obese (BMI ≥40 kg/m^2). Exclusion criteria were: liver and kidney diseases and neoplasms.

The control group consisted of 25 healthy subjects aged 57.6±11.9 years with a normal body mass index (BMI <25 kg/m^2) who reported for the routine health checks to the Department of Laboratory Diagnostics SPSK No. 1 in Lublin. The control group consisted of 14 women aged 57.1±12.3 and 11 men, aged 58.3±12.1 years.

All patients underwent measurements of height and weight using the calibrated equipment. Measurements were performed with precision of 1cm for the height and 0.1 kg for the body weight. Based on this results BMI was calculated according to the formula BMI= body weight (kg)/body height (m^2) (kg/m^2) [26].

All patients consented to the study after information of the nature of the research was explained.

METHODS

Morning (8-10 am) venous blood samples were drawn from each subject after 8 hours or overnight fasting into 5 ml K_3EDTA tubes for HbA1c determination and to the clotted tube for other biochemical parameters and selected bone markers. Tubes were left to clot for about 30 minutes then centrifuged for 10 minutes at 2000 rpm. Obtained serum was separated to the eppendorf tubes and stored frozen at -20°C until assayed.

The measurements of OCN and CTx as well as glucose (GLU), total cholesterol (T-CH), HDL cholesterol (HDL-CH), triglycerides (TG), urea, creatinine, parathyroid hormone (PTH), glycosylated hemoglobin A1c (HbA1c) concentrations were performed in all patients. Moreover, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were measured. Biochemical parameters listed above were determined with the use of standard laboratory methods applied on Cobas 6000 analyzer (Roche, Basel, Switzerland) with dedicated reagents from the same company according to the manufacturer's specification. PTH concentration in the serum was performed with the use of electrochemiluminescence method on Cobas e411 analyzer (Roche, Basel, Switzerland) using ready kits provided by Roche. LDL cholesterol (LDL-CH) level was calculated with use of the Friedewald formula: LDL-CH= [T-CH] – [HDL-CH] – [TG/5] [8].

Commercially available enzyme-linked immunosorbent assay kits (ELISA) were used to determine OCN (N-MID Osteocalcin ELISA) and CTx (Cross Laps ELISA) serum concentrations (Nordic Bioscience Diagnostics, Denmark).

The inter-assay coefficient of variation for the N-MID Osteocalcin ELISA was 3.6% at a concentration of 6.8 ng/ml, 6.4% at 50.5 ng/ml, while intra-assay CV% was 3.4% at 7.0 ng/ml and 2.0% at a concentration of 21.8 ng/ml. Analytical sensitivity of the assay was: 0.5 ng/ml.

The inter-assay coefficient of variation for the Cross Laps ELISA was 8.1% at a concentration of 0.273 ng/ml, 5.4% at 0.393 ng/ml, while intra-assay CV% was 5.4% at 0.242 ng/ml and 5.0% at a concentration of 0.375 ng/ml. Sensitivity of Cross Laps assay was 0.020 ng/ml.

Statistical analysis. All statistical analyses were conducted with the StatSoft STATISTICA 10.0 statistical package. All variables in study group and controls are shown as elements of descriptive statistics (arithmetic mean (χ), standard deviation (SD), median (Me) percentile range (25-75%). Distribution of the variables was tested using the Shapiro-Wilk's test. Normally distributed variables were compared with use Student's t-test, and variables with skewed distribution were compared with use of nonparametric Mann-Whitney U test. The correlations between variables were appropriately tested using Pearson or Spearman tests. Statistical significance for all analyses was set at $p \leq 0.05$.

RESULTS

The biochemical characteristics of the study group and the controls are presented in Table 1.

The average concentration of glucose in the serum of DM2 patients was 168.4±61.6 mg/dl and was significantly higher ($p<0.0001$) than in healthy subjects (85.2±9.3

mg/dl). Significantly higher levels of urea, creatinine, TG ($p<0.0001$) and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ($p<0.05$) were found in patients with DM2 in comparison with healthy subjects. Additionally HDL cholesterol was significantly lower ($p<0.0001$) in the study group than in the control group. Among the parameters of lipid profile the total cholesterol and CH-LDL did not show significant differences between DM2 patients and healthy controls. Mean HbA1c level in the study group was 8.6±2.1%.

The concentrations of selected markers of bone turnover in the study group and controls are presented in Table 2.

In DM2 patients the concentrations of OCN (14.5±8.8 ng/ml) and PTH (23.9±14.5 pg/ml) were significantly lower ($p<0.001$) than in healthy subjects (28.1±11.9 ng/ml and 40.0±13.2 pg/ml for OCN and PTH, respectively). The average concentration of CTx in the study group was 0.3±0.2 ng/ml and was significantly lower ($p<0.05$) than in controls (0.5±0.2 ng/ml). There were no significant differences in ALP activity between DM2 patients and healthy controls.

The concentrations of biochemical parameters and selected bone turnover markers depending on gender in the study group of DM2 patients are shown in Table 3.

Significantly higher levels of OCN ($p<0.05$) and CTx ($p<0.01$) were found in women than in men within the study group. The concentration of OCN and CTx in women was 17.4±9.9 ng/ml and 0.4±0.3 ng/ml respectively, while in men 11.8±6.7 ng/ml and 0.3±0.2 ng/ml, respectively. A similar statistically significant difference was observed in the concentration of PTH and HDL cholesterol. Other biochemical parameters did not show significant differences between men and women with DM2.

Table 1. Biochemical characteristics of the study and the control group

Parameters	Study Group n=45			Control Group n= 25		
	χ ±SD	Me	25-75%	χ ±SD	Me	25-75%
Glucose (mg/dl)	168.4±61.6‡	156.0	132.0-192.0	85.2±9.3	87.0	79.0-92.0
HbA1c (%)	8.6±2.1	8.0	7.2-9.7	-	-	-
T-CH (mg/dl)	190.1±46.7	183.0	155.0-213.0	196.1±29.1	205.0	183.0-217.0
CH-HDL (mg/dl)	44.0±10.1‡	43.0	37.0-49.0	57.3±13.1	57.6	50.0-63.0
CH-LDL (mg/dl)	112.9±41.9	101.8	81.4-140.0	119.6±28.3	123.4	106.2-140.4
TG (mg/dl)	191.4±91.3‡	174.0	121.0-237.0	96.5±41.1	89.0	63.0-118.0
Urea (mg/dl)	36.4±12.9‡	34.7	28.6-41.4	33.6±7.8	30.3	28.0-39.0
Creatinine (mg/dl)	0.9±0.2‡	0.9	0.8-1.0	0.7±0.1	0.7	0.7-0.8
ALT (U/l)	31.9±19.2†	25.0	20.0-37.0	20.6±9.2	19.0	14.3-23.8
AST (U/l)	26.9±12.7†	23.0	20.0-29.0	21.2±5.0	20.0	18.2-23.5

n – number of subjects
† $p<0.05$
‡ $p<0.0001$

Table 2. Concentration of selected bone turnover markers in the study and the control group

Parameters	Study Group n=45			Control Group n= 25		
	χ ±SD	Me	25-75%	χ ±SD	Me	25-75%
OCN (ng/ml)	14.5±8.8‡	11.8	8.8-17.1	28.1±11.9	26.3	19.4-35.4
CTx (ng/ml)	0.3±0.2†	0.3	0.2-0.5	0.5±0.2	0.4	0.3-0.5
ALP (U/l)	63.1±16.9	61.0	52.0-73.0	70.3±18.9	67.0	54.0-85.0
PTH (pg/ml)	23.9±14.5‡	20.6	14.5-26.6	40.0±13.2	36.2	31.3-48.7

n – number of subjects
† $p<0.05$
‡ $p<0.001$

Table 3. The concentrations of biochemical parameters and selected bone turnover markers in female and men in the study group of DM2 patients

Parameters	Study group n=45					
	Women n=22			Men n=23		
	χ ±SD	Me	25-75%	χ ±SD	Me	25-75%
Glucose (mg/dl)	160.0±56.9	155.5	116.0-180.0	176.4±66.1	160.0	132.0-192.0
HbA1c (%)	8.5±2.4	7.9	6.8-8.7	8.7±1.8	9.0	7.3-9.8
T-CH (mg/dl)	196.2±45.3	186.0	164.0-231.0	184.2±48.3	183.0	146.0-212.0
CH-HDL (mg/dl)	47.9±11.1†	44.0	42.0-52.0	40.2±7.4	40.0	35.0-44.0
CH-LDL (mg/dl)	114.7±41.7	107.9	81.4-151.6	111.2±43.0	101.8	73.6-136.0
TG (mg/dl)	117.6±82.8	161.0	115.0-237.0	204.7±98.7	191.0	121.0-287.0
Urea (mg/dl)	37.6±15.6	36.2	26.7-42.5	35.2±9.7	33.4	29.0-38.9
Creatinine (mg/dl)	0.9±0.2	0.9	0.7-0.9	0.9±0.1	0.9	0.9-1.0
ALT (U/l)	28.5±15.7	23.0	19.0-34.0	35.1±22.0	29.0	20.0-38.0
AST (U/l)	27.0±16.1	22.0	20.0-27.0	26.8±8.7	24.0	21.0-33.0
OCN (ng/ml)	17.4±9.9†	15.2	9.9-23.4	11.8±6.7	10.4	7.8-14.8
CTx (ng/ml)	0.4±0.3‡	0.4	0.2-0.5	0.3±0.2	0.2	0.2-0.3
ALP (U/l)	67.3±15.7	66.0	55.0-77.0	58.9±17.4	55.0	48.0-68.0
PTH (pg/ml)	30.7±17.7‡	24.1	17.8-39.0	17.5±6.0	15.8	12.7-22.2

n – numer of subjects
† $p<0.05$
‡ $p<0.01$

The correlations between the OCN, CTx and basic demographic and anthropometric characteristics as well as selected biochemical parameters in the study group are presented in Table 4.

Table 4. The correlation of OCN and CTx with the basic demographic and anthropometric characteristics and selected biochemical parameters in the study group

Parameters	Study group n=45			
	OCN (ng/ml)		CTx (ng/ml)	
	Correlation coeff. r	p value	Correlation coeff. r	p value
Age (years)	0.239	0.114	0.196	0.196
Body weight (kg)	-0.110	0.474	-0.208	0.171
BMI (kg/m^2)	0.055	0.720	-0.029	0.852
Disease duration (years)	-0.001	0.995	-0.061	0.692
OCN (ng/ml)	–	–	0.721	**0.000**
CTx (ng/ml)	0.721	**0.000**	–	–
ALP (U/l)	0.248	0.109	0.396	**0.009**
PTH (pg/ml)	0.426	**0.003**	0.413	**0.005**
Glucose (mg/dl)	-0.216	0.155	-0.192	0.206
HbA1c (%)	-0.267	0.077	-0.349	**0.019**
T-CH (mg/dl)	-0.076	0.618	-0.099	0.520
CH-HDL (mg/dl)	0.243	0.107	0.144	0.346
CH-LDL (mg/dl)	-0.038	0.804	-0.024	0.875
TG (mg/dl)	-0.349	**0.019**	-0.302	**0.044**
Urea (mg/dl)	0.034	0.827	0.071	0.640
Creatinine (mg/dl)	0.012	0.940	-0.002	0.991
ALT (U/l)	-0.163	0.286	0.035	0.817
AST (U/l)	-0.135	0.378	-0.112	0.642

n – numer of subjects
p – level of statistical significance ($p<0.05$)

In D2 patients OCN concentration was significantly directly correlated with CTx (r=0.721, p=0.000) and PTH (r=0.426, p=0.003). Additionally, there was significant negative correlation between OCN and the TG values (r=-0.349, p=0.019). Significant positive correlations was found between serum CTx and ALP values (r=0.396, p=0.009) and PTH (r=0.413, p=0.005) and a negative correlation between serum CTX and TG (r=-0.302, p=0.044) as well as HbA1c (r=-0.349, p=0.019). No significant correlations were found in the control group between OCN and/or CTx and other biochemical parameters evaluated in this study.

DISCUSSION

Vast of literature indicates skeletal involvement in patients with diabetes mellitus type 2. Bone alterations usually develop after many years of diabetes, but it may also occur in the early stages of the disease [1,4,20,24]. The endocrine and metabolic disturbances in DM2 trigger changes in calcium homeostasis, skeletal metabolism and bone mass [14]. Different elements of the skeleton can be affected as bones, joints and periarticular tissues; primarily due to excessive collagen deposition in subcutaneous and periarticular tissue. One of the causes may be an increased vascular permeability, which stimulates the proliferation of periarticular tissues as well as excessive activity of various growth factors. In diabetes in the excess of glucose, proteins undergoes the advanced non-enzymatic glycation (AGE) which leads to an increased crosslinking of collagen which are resistant to collagenase hence reduction of collagen turnover is observed as well as the accumulation of collagen fibers in the tissues. The amount of AGE products of bone collagen increases due to its impaired degradation in diabetes. This acumulation leads to decreased bone strength and increase bone fragility [11,23-25]. Wang X. et al. [25] found that a higher concentration of AGEs was associated with decreased strength. Many other studies confirmed that diabetes itself is associated with increased risk of fracture of the hip, proximal humerus, and foot [3,10,14,24].

Significant advances in research on biochemical markers of bone turnover have been achieved in recent years, which allow the quick and non-invasive assessment of bone formation and resorption processes. The role of bone turnover markers is recognized in monitoring bone mass changes in the course of treatment as well in prediction of the occurrence of bone fractures, including in DM2 patients [1,4,18,24].

Among all bone turnover markers the concentration of OCN is most often used as a marker of formation, and cross-linked telopeptides of type I collagen (NTx and CTx) as a markers of resorption [1,2,18,20,24].

In the present study we demonstrated significantly reduced levels of OCN ($p<0.001$) and CTx ($p<0.05$) in the serum of patients with type 2 diabetes in comparison to controls. This results are consistent with reports by others, who emphasized the role of slow bone turnover in patients with DM2 as a result of insufficient formation process due to defects in osteoblasts maturation. This results in reduction of markers osteogenesis in serum [1,4,10,14,20,24]. Other studies also suggests that the process of bone resorption in type DM2 is normal [1,10] or relatively increased as compared to decreased bone formation [14,20] and rarely has been shown to be reduced [2,4,13,24], as demonstrated in this study. The study conducted by Oz S.G. et al. [14] has shown decreased bone turnover in DM2 with reduced bone formation processes and increased resorption, what was accompanied by reduced levels of OCN and increased urinary excretion of markers of resorption. Also, the study performed by Akin O. et al. [2] confirmed remarkably lower bone turnover rate in DM2 patients compared to healthy postmenopausal patients.

Bone turnover markers in the study performed by Trznadel-Morawska I. et al. [22] were evaluated in respect to the dynamics of bone changes as well as markers of prediction of bone alterations in patients with DM1 and DM2 with diabetic foot. They found that diabetics patients had lower levels of markers of bone formation (bone alkaline phosphatase, OCN), and elevated levels of markers of resorption (NTx) additionally to lower bone mass. This overall indicates the reduction in process of bone formation and increase in resorption of in these patients.

Alkaline phosphatase (ALP) despite the lack of tissue specificity is routinely analyzed marker which reflects bone formation once liver diseases are ruled out [18,24]. Significant decrease in the activity of ALP bone isoenzyme as well as in the total ALP activity in patients with DM2 has been reported already by others [13,14]. We also observed lower ALP activity in the DM2 group in comparison to the control group, however, this difference did not reach statistical significance. In our study, a positive correlation was observed between serum CTx and the activity of ALP in patients with DM2. A similar relationship has been demonstrated with respect to the concentration of OCN and CTx. It may be related to the maintenance of the constant bone mass in these patients.

In our study we demonstrated significantly lower ($p<0.001$) concentrations of parathyroid hormone (PTH) in the study group than in the controls. In addition, the concentrations of OCN and CTx in patients with DM2 correlated positively with serum PTH, which probably results from the preserved loop between formation and bone resorption. PTH receptors acting on osteoblasts and bone marrow stromal cells can indirectly stimulate bone resorption. It increases the synthesis and release of proreceptive cytokines: IL-1, IL-6 and M-CSF as well as stimulates the RANKL gene expression in osteoblasts and bone marrow stromal cells additionally, may also inhibit the apoptosis of osteoclasts. The decrease in PTH levels in patients with DM2 may influence whether bone mass is maintained or increased in these patients [9,24,27].

In our study, women with DM2 showed significantly higher levels of OCN and CTx than men, which seems to be supported by available literature [2,3,14]. The increase in bone turnover markers suggests intensification of the resorption processes of increase bone turnover in women than in men.

It seems to be that fat and muscle mass play an important role in determining the bone mass density [16]. The protective effect of high BMI on bone has been proven by De Leat C. et al. [6]. In published meta-analysis of 12 studies involving nearly 60,000 people of both genders he showed that the risk of proximal femur fracture decreases by 93% for every kg/m^2 increase in BMI. In addition, there is evidence of a negative relationship between body weight and the activity of osteoclasts, so widespread knowledge is that obesity protects against bone fractures [16,17]. We also showed a negative correlation between serum CTx marker of osteoclast activity and body weight and BMI in patients with DM2, although it was not statistically significant.

It is well known that chronic hyperglycemia leads to both quantitative and qualitative changes in lipid profile. It is estimated that lipid disturbances can be found in nearly 60-80% DM2 patients. Most frequently it is atherogenic dyslipidemia characterized by elevated TG, low HDL-cholesterol levels and the presence atherogenic LDL (so-called of small, dense LDL particles) within LDL subtractions [12]. In our study we found a significantly lower ($p<0.0001$) HDL cholesterol as well as significantly higher ($p<0.0001$) TG levels in patients with DM2 than in healthy subjects. Interestingly, we found a statistically significant inverse correlation between the values of TG and CTx ($r=-0.302$, $p=0.044$) and OCN ($r=-0.349$, $p=0.019$).

The mechanism of adverse impact of lipids on bone is not yet fully understood hence it is difficult to explain inverse correlation between OCN and CTx levels with TG levels found in this study. Plausible explanation of adverse effect of oxidized lipids on bone would be to inhibit osteoblast differentiation on one hand and increase the recruitment and differentiation of osteoclasts on the other, leading further to excessive bone resorption and osteoporosis [15].

The role of control of metabolic disturbances in the maintenance of bone mass in patients with DM2 should be also emphasized. Vast literature reports that bone disturbances are often observed in patients with poor metabolic control. It has been shown that end products of protein glycation tend to hinder the function of osteoblasts. Patients with non-adequate compensation of DM2 as well as type 1 diabetes (DM1) were more prone to bone loss [4,24]. Okazaki R. et al. [13] found that the restoration of good metabolic control of diabetes inhibits in the short term loss of bone mass and leads to stabilization of bone mineral density. In the period of relative diabetes compensation it is more likely that the factors dependent on obesity and fat mass predominate, what further stimulate bone formation. Achemlal L. et al. [1] found a negative correlation between serum CTx and the level of HbA1c, suggesting increased bone turnover in patients with DM2 and improved glycemic control. This study confirmed presence of such relationship.

Yendt E.R. et al [28] demonstrated a direct correlation between values of creatinine clearance and the bone density and bone mass. In the present study we demonstrated significantly higher ($p<0.0001$) serum creatinine and urea levels in patients with DM2 than in healthy subjects. However we did not find significant correlations neither with CTx nor OCN concentrations.

Clinical usefulness of bone markers is well documented and accepted by clinicians of many specialties. Determination of biochemical markers of bone turnover may be useful in monitoring the course of various diseases affecting the bone, as well as in predicting the effects of the treatment. It should be highlighted that the biochemical markers of bone turnover are considered as risk factors for fracture independently of bone mineral density. However it needs further validation to accurately determine their practical importance in a variety of medical disci-

plines, including utility in monitoring bone changes in patients with DM2.

CONCLUSIONS

1. In obese DM2 patients lower concentrations of OCN and CTx are observed in comparison to healthy subjects with normal body weight.
2. Higher concentrations of OCN and CTx were found in women than in men with DM2.
3. The observed lower levels of OCN and CTx and their positive inter-correlation may suggest overall slowed down bone metabolism with reduced bone formation and bone resorption in patients with DM2.

REFERENCES

1. Achemlal L. et al.: Bone metabolism in male patients with type 2 diabetes. *Clin. Rheumatol.,* 24, 493, 2005.
2. Akin O. et al.: Evaluation of bone turnover in postmenopausal patients with type 2 diabetes mellitus using biochemical markers and bone mineral density measurements. *Gynecol. Endocrinol.*, 17(1), 19, 2003.
3. Bonds D.E. et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J. Clin. Endocrinol. Metab*., 91, 3404, 2006.
4. Capoglu I. et al.: Bone turnover markers in patients with type 2 diabetes and their correlation with glycosylated haemoglobin levels. *J. Int. Med. Res.*, 36, 1392, 2008.
5. DeFronzo R.A: Pathogenesis of type 2 diabetes mellitus. *Med. Clin. N. Am.,* 88, 787, 2004.
6. De Laet C. et al.: Body mass index as a predictor of fracture risk: a meta-analysis. *Osteop. Int.*, 16, 1330, 2005.
7. Dennison E.M. et al.: Type 2 diabetes mellitus is associated with increased axial bone density in men and woman from the Hertfordshire Cohort Study: evidence for an indirect effect of insulin resistance? *Diabetologia*, 47, 1963, 2004.
8. Friedewald W.T., Levy R.I., Fredrickson D.S.: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem*., 18, 499, 1972.
9. Hadjidakis D.J., Androulakis I.I.: Bone remodeling. *Ann. N.Y. Acad. Sci.*, 1092, 385, 2006.
10. Leidig-Bruckner G., Ziegler R.: Diabetes mellitus a risk for osteoporosis? Exp. Clin. Endocrinol. *Diabetes*, 109(2), 493, 2001.
11. McCarthy A.D., Etcheverry S.B., Cortizo A.M.: Effect of advanced glycation endproducts on the secretion of insulin-like growth factor-I and its binding proteins: role in osteoblast development. *Acta Diabetol.*, 38, 113, 2001.
12. Mooradian A.D.: Dyslipidemia in type 2 diabetes mellitus. *Nat. Clin. Pract. Endocrinol. Metab*., 5(3), 150, 2009.
13. Okazaki R. et al.: Metabolic improvement of poorly controlled noninsulin-dependent diabetes mellitus decreases bone turnover. *J. Clin. Endocrinol. Metab*., 82, 2915, 1997.
14. Oz S.G. et al.: Evaluation bone metabolism and bone mass in patients with type 2 diabetes mellitus. *J. Natl. Med. Assoc.*, 98, 1598, 2006.
15. Parhami F., Garfinkel A., Demer L.L.: Role of lipids in osteoporosis. *Arterio. Thromb. Vasc. Biol.*, 20(11), 2346, 2000.
16. Reid I.R.: Relationships between fat and bone. *Osteoporos Int*., 19, 595, 2008.
17. Revilla M. et al.: Influence of body mass index on the age-related slope of total and regional bone mineral content. *Calcif. Tissue Int.*, 61, 134, 1997.
18. Srivastava A.K. et al.: Clinical use of serum and urine bone markers in management of osteoporosis. *Curr. Med. Res. Opin*., 21, 1015, 2005.
19. Steyn N.P. et al.: Diet, nutrition and the prevention of type 2 diabetes. *Public Health Nutr*, 7(1A), 147, 2004.
20. Suzuki K. et al.: Osteoclastic function is accelerated in male patients with type 2 diabetes mellitus: the preventive role of osteoclastogenesis inhibitory factor/osteoprotegerin (OCIF/OPG) on the decrease of bone mineral density. *Diab. Res. Clin. Pract.*, 68, 117, 2005.
21. Thrailkill K.M. et al.: Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. *Am. J. Physiol. Endocrinol. Metab*., 289, 735, 2005.
22. Trznadel-Morawska I. et al.: Przydatność markerów obrotu kostnego w dynamice zmian kostnych w zespole stopy cukrzycowej. *Diabetol. Prakt*., 9(2), 63, 2008.
23. Vashishth D. et al.: Influence of nonenzymatic glycation on biochemical properties of cortical bone. *Bone*, 28, 195, 2001.
24. Vestergaard P.: Bone metabolism in type 2 diabetes and role of thiazolidinediones. *Curr. Opin. Endocrinol. Diabetes Obes*., 16, 125, 2009.
25. Wang X. et al.: Age-related changes in the collagen network and toughness of bone. *Bone*, 31(1), 1, 2002. 15
26. World Health Organization: Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech. Rep. Ser.,* 854, 1, 1995.
27. Yamamoto M. et al.: Decreased PTH levels accompanied by low bone formation are associated with vertebral fractures in postmenopausal women with type 2 diabetes. *J. Clin. Endocrinol. Metab*., 97(4), 1277, 2012.
28. Yendt E.R. et al.: Reduced creatinine clearance in primary osteoporosis in women. *J. Bone Miner. Res*., 8, 1045, 1993.