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CHANGES IN BONE MINERAL DENSITY IN YOUNG ADULTS WITH TYPE 1 DIABETES MELLITUS

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Abstract

Background and aims*: Type 1 diabetes mellitus (T1DM) represents a secondary cause of osteoporosis. Our aim was to determine bone mineral density (BMD) changes in a group of young Romanian adults with T1DM and to analyze the factors related to this disease that could have had an impact on bone mass.* ***Material and Methods****: Fifty-two young patients with T1DM were compared to 37 healthy volunteers matched for body mass index (BMI). All subjects had their BMD measured at the hip and lumbar spine.* ***Results****: We found no statistically significant differences in BMD between T1DM patients and controls (p=0.618 for lumbar spine, p=0.974 for femoral neck and p=0.883 for total hip). Multiple linear regression models detected BMI (p =0.043), smoking (p=0.001) and milk intake (p=0.004 for lumbar spine) as significant BMD determinants. In contrast, no associations were found between BMD and metabolic control, daily insulin dose or presence of diabetic retinopathy and/or neuropathy. Long diabetes duration was negatively associated with BMD in femoral neck (p=0.012).* ***Conclusions****: Although we couldn't find differences between BMD in T1DM patients and controls, the link between diabetes duration and BMD that we found suggests that even young patients with long standing T1DM should have their BMD measured.*

key words: *osteoporosis, bone mineral density, type 1 diabetes mellitus*

Background and Aims

Type 1 diabetes mellitus (T1DM) is considered to be a secondary cause of osteoporosis, patients with T1DM having a high risk of fracture due to decreased bone mineral density (BMD), impairment of bone quality and extra skeletal factors that increase the probability of falls [1-5].

In the past years, a number of studies have focused on determining BMD in T1DM patients from various age groups (children, adolescents, young adults and older patients) with conflicting results. Many of these studies reported a lower

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BMD in T1DM patients compared with healthy subjects [6-11], while there are a number of studies that did not report modifications of BMD in T1DM patients [12,13]. Assuming the low BMD of T1DM patients, questions were raised regarding the causal factors. Several factors were mentioned as possibly involved in BMD modification, including diabetes duration, disease control and presence of diabetes chronic complications. Regarding diabetes duration, opinions are contradictory, some said it is associated with low BMD [11,12,14], others found the contrary [13,15,16]. Poor disease control may have an effect on BMD [8,9,12,14] or not [15-18]. The same happens with diabetes neuropathy [10,19,20] and microvascular complications [2,17,18,21]. Low BMD is associated with high daily insulin doses, the presence of chronic complications regardless of their type and particularly with diabetic nephropathy [2,22,23]. As in other states of bone loss, low levels of IGF-1 observed in T1DM could impact on bone mass [24-26]. Age at diagnosis, microalbu-minuria, retinopathy and macrovascular complications were not proved to influence BMD [7,15,16].

In view of the previous conflicting data, the aim of our study was to determine the BMD in a group of young Romanian adults with T1DM as well as to identify the factors possibly associated with low BMD.

Material and Methods

Our research was carried out as an analytic transversal, case-control study. Fifty-two young patients with T1DM (29 men and 23 women), aged 20 to 55, were included in the study between April 2011 and April 2012. These patients were enrolled from the patients followed at the Clinical Center of Diabetes, Nutrition and Metabolic Diseases Cluj-Napoca that fulfilled all the inclusion criteria and had no exclusion criteria. Inclusion criteria were: men or premenopausal women aged 20 to 55, with previous diagnosis of T1DM. Exclusion criteria were: confirmed diabetic nephropathy (patients with transient microalbuminuria were accepted), postmenopausal status, presence of other secondary causes of osteoporosis (e.g. endocrine pathologies, gastro-intestinal diseases, rheuma-tologic conditions, drugs, as described elsewhere [27]). The patients were compared to 37 healthy volunteers (control group), matched (as group mean) for age and body mass index (BMI). Inclusion criteria were: non-diabetic subjects (men and premenopausal women), aged 20 to 55. Exclusion criteria were the same as those used for diabetic patients. All premenopausal women enrolled (patients and controls) had a normal puberty onset and regular menses and fulfilled all the inclusion criteria. The study was carried out according to Helsinki Declaration. All subjects signed an informed consent prior to inclusion in the study. The study was approved by the Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca. Clinical status of the study participants was evaluated using a questionnaire that was administered by the study physician and was used to collect information regarding personal history (diseases and medication), personal and parental history of fracture, personal trauma history, smoking status, alcohol and coffee consumption, physical activity and sun exposure. According to smoking habits, the participants were divided into three categories: current smokers, ex-smokers (patients with an abstinence period of at least 6 months), non-smokers /never smokers. Alcohol consumption over the last year (qualitative evaluation) was divided into frequent (three or more times a week), occasional (twice a week or less) and none (the patient denies consuming alcohol). Coffee consumption in the last year was assessed qualitatively as consumer or non-consumer. Physical activity evaluation

categories were: vigorous (one hour four times a week of jogging, gymnastics, fitness or heavy duty work daily at house or job), moderate (one hour two or three times a week of jogging, gymnastics, fitness or daily walking more than two kilometers to work, at work or moderate activity as home labor), low (walking less than two kilometers daily, easy home labor or a job standing/easy and walking), sedentary (spending most of the time sitting at work and home, with hobbies not implicating physical exercise). Sun exposure was sectioned into three categories: frequent (deliberately searching for sun in summer and spring, using tanning beds or booths or work involving daily sun exposure in summer and spring), moderate (deliberate exposure to sun only in vacations with work not involving sun exposure), and low (no deliberate sun exposure, avoiding exposure in summer or spring). Additionally, milk products (milk and powder milk, cream, yogurt, cheese and derivatives) intake over the last year was evaluated qualitatively in four categories: every day (6-7 times a week), weekly (2-5 times a week), occasional (once a week or less), avoids (the patient avoids consuming milk products). Calcium, magnesium and vitamin D supplements consumption over the last six months were each evaluated qualitatively (consumer or non-consumer).

An anthropometric assessment was performed. Standing height was measured using a calibrated stadiometer and for weight determination a calibrated analog scale was used. Abdominal and hip circumferences were tape measured. Systemic arterial blood pressure was measured using a stethoscope and a sphygmomanometer. Systolic and diastolic values were expressed in millimeters of mercury (mmHg). BMI calculation formula was the weight (kilograms) divided by height (meters) squared [28].

Additional information regarding patients with T1DM was collected from existing files. Data were collected regarding chronic complications, diabetes control (HbA1c glycated hemoglobin history) and biological parameters assessed at their last evaluation. Serum total cholesterol, HDL cholesterol, triglycerides, ASAT and ALAT were determined by an enzymatic colorimetric method using commercially available Diagnosticum Inc. (Budapest, Hungary) kits. Serum LDL was calculated using the Friedewald formula: serum LDL cholesterol equal to serum total cholesterol minus serum HDL cholesterol minus serum triglycerides divided by 5. Serum creatinine was measured using a colorimetric, alkaline picrate method (Jaffé) with a kit from Diagnosticum Inc. (Budapest, Hungary). HbA1c (HbA1c last value) was determined using an immunoturbidimetric assay test: Tina-quant Hemoglobin A1c Gen. 2 kit from Roche Diagnostics GMBH (Mannheim, Germany). HbA1c history values available since the diagnosis of diabetes were taken from the patient's file. Regarding T1DM chronic complications, ischemic cardiac disease and cerebrovascular disease were considered when there existed personal history of angina pectoris or myocardial infarction (or documented evidence of ischemic coronary artery disease from a cardiologic examination) and respectively transient ischemic attack or stroke. Peripheral arterial disease was diagnosed with Doppler ultrasound (ankle brachial index). Diabetic retinopathy was assessed with direct ophthalmoscopic examination (ophthalmologic exam). The presence of diabetic neuropathy was evaluated using standard methods: by testing vibration perception with a calibrated tuning fork, by testing pressure sensation with a 10 g calibrated Semmes-Weinstein monofilament, by testing tactile sensitivity with cotton pads, needle testing for sensitivity to pain and assessment of osteotendinous reflexes. Microalbuminuria was

defined as albuminuria of 30-300 mg/24h or 20-200 µg/min. Transient microalbuminuria was defined as only one positive sample followed by negative ones [29].

Table 1. Clinical characterization of the diabetes mellitus type 1 patients group.

	Females	Males	P-value
Number	23	29	-
Age (years)	27 [24 - 33]	32 [26 - 42]	0.123
Diabetes duration (years)	12 [8 - 16.5]	12 [3 - 18]	0.810
BMI (kg/m^2)	22.98 [21.42 - 25.55]	23.56 [20.76 - 26.59]	0.651
Waist-hip ratio	0.81 [0.76 - 0.87]	0.88 [0.85 - 0.91]	0.0009
Weight variation (last year) (kg)	0 [0 - 1]	0 [0 - 0]	0.400
Systolic blood pressure (mmHg)	120 [110 - 130]	130 [120 - 140]	0.035
Diastolic blood pressure (mmHg)	72 [70 - 80]	80 [75 - 90]	0.002
Ischemic cardiac disease *Angina pectoris* *Myocardial infarction* *None*	 0 (0 %) 1 (4.34 %) 22 (95.65 %)	 1 (3.44 %) 0 (0 %) 28 (96.55 %)	0.693
Cerebro-vascular disease	0 (0 %)	1 (3.448 %)	1
Peripheral arterial disease	0 (0 %)	0 (0 %)	-
Neuropathy	4 (17.39 %)	7 (24.15 %)	0.554
Retinopathy	6 (26.08 %)	10 (34.48 %)	0.514
Transient microalbuminuria	3 (13.04 %)	1 (3.44 %)	0.310
Parental history of fracture	4 (17.39 %)	8 (27.58 %)	0.386
Personal history of fracture	0 (0 %)	1 (3.44 %)	1
Personal trauma history	5 (21.73 %)	5 (17.24 %)	0.734
Smoking status *Ex-smoker* *Current smoker* *Non-smoker*	 2 (8.69 %) 10 (43.47 %) 11 (47.82 %)	 3 (10.34 %) 13 (44.82 %) 13 (44.82 %)	1
Alcohol consumption *Denies* *Occasional* *Frequent*	 9 (39.13 %) 14 (60.87 %) 0 (0 %)	 5 (17.24 %) 22 (75.86 %) 2 (6.89 %)	0.109
Coffee consumption	19 (82.6 %)	25 (86.2 %)	1
Physical activity *Vigorous* *Moderate* *Low* *Sedentary*	 2 (8.69 %) 10 (43.47 %) 10 (43.47 %) 1 (4.34 %)	 4 (13.79 %) 15 (51.72 %) 8 (27.58 %) 2 (6.89 %)	0.719
Sun exposure *Frequent* *Moderate* *Low*	 0 (0 %) 13 (56.52 %) 10 (43.47 %)	 3 (10.34 %) 14 (48.27 %) 12 (41.37 %)	0.390
Milk products intake *Avoids* *Occasional* *Weekly* *Every day*	 0 (0 %) 4 (17.39 %) 11 (47.82 %) 8 (34.78 %)	 3 (10.34 %) 4 (13.79 %) 12 (41.37 %) 10 (34.48 %)	0.526
Calcium supplements	4 (17.39 %)	5 (17.24 %)	1
Magnesium supplements	6 (26.08 %)	6 (20.69 %)	0.646
Vitamin D supplements	2 (8.69 %)	0 (0 %)	0.190
Key: qualitative data: n (%), n=number of patients, %=percentage; not normally distributed quantitative data: median [quartile1-quartile3];			

All subjects had their BMD measured using dual-energy X-ray absorptiometry (DXA) in two areas of interest: hip and lumbar spine (L1-L4). Hip evaluation assessed two self–explanatory areas, femoral neck and trochanteric area, the total hip area being a sum of these two areas. A DPX-NT (GE, Madison, USA) equipment was used [30]. The precision error for TBFM (total

body fat mass) was <3%. The DXA equipment was calibrated on a regular basis using the phantom provided by the manufacturer. The investigation was performed at the Clinic of Endocrinology Cluj-Napoca.

Statistical analysis

Quantitative data was presented as mean and standard deviation for normally distributed variables, and median with interquartile range for not normally distributed variables. Normality of the data was checked with quartile-quartile plot and Shapiro-Wilk test. Qualitative data was presented by number and percentages and the association between qualitative variables was tested using Fisher exact test, if more than 20% of expected frequencies were less than 5 or Chi square test otherwise. Comparisons between two groups regarding quantitative variables were made with Student t test for independent samples for normally distributed variables and Mann Whitney U test for not normally distributed data. Simple linear regression was used to assess relationships of different variables with BMD, and then multiple linear regression was performed to adjust for known important predictors. For inclusion in the linear regression as independent variable, categorical variables were recoded as dummy variables (were assigned values 0 or 1). For all statistical tests used, the significance level alpha chosen was 0.05, and the two tailed p value was computed. The statistical analysis was made in R ENVIRONMENT for statistical computing and graphics, version 1.15.1 [31].

Results

Characterization of T1DM group. T1DM patients group had a median age of 28.5 [24.75-38.25] years. Median for last value of glycated hemoglobin was 7.85 [7.10-8.60] %. Median diabetes duration was 12 [4.75-18] years. Regarding diabetes chronic complications, two patients had ischemic cardiac disease (3.84 %), one had cerebrovascular disease (1.92 %), eleven patients had neuropathy (21.15 %) and sixteen had retinopathy (30.77 %). No patient presented peripheral arterial disease. Characteristics of patients with T1DM are presented in Tables 1 and 2. Waist-hip ratio, systolic and diastolic blood pressure, liver enzymes and serum creatinine values were statistically significant higher in men than in women (p <0.05 in all). All other variables presented similar values both in men and women.

Table 2. Biochemical characterization of the diabetes mellitus type 1 patients group.

	Females	Males	P-value
Number	23	29	-
Serum total cholesterol (mg/dl)	179 [162 - 193.5]	168.5 [153.25 - 189.75]	0.351
Serum HDL cholesterol (mg/dl)	62 [42.5 - 67.5]	47 [40.5 - 57]	0.072
Serum LDL cholesterol (mg/dl)	105.2 [90.9 - 125.4]	101.7 [91.85 - 115.97]	0.541
Serum triglycerides (mg/dl)	75 [56.5 - 91.5]	86 [59 - 104]	0.242
ASAT (U/l)	15 [12.5 - 17.5]	22 [17.25 - 31]	0.0004
ALAT (U/l)	15 [12.5 - 20.5]	23.5 [17.17 - 27]	0.001
Serum creatinine (mg/dl)	0.78 [0.72 - 0.84]	0.925 [0.8 - 1.03]	0.003
Creatininic clearance (ml/min) (Cockcroft-Gault Equation)	104.74 [97.44 - 112.29]	118.29 [97.65 - 136.06]	0.126
HbA1c last value (%)	7.8 [7.15 - 8.05]	7.9 [7.1 - 9.1]	0.366
HbA1c last year (%)	8.16 [7.41 - 8.45]	7.8 [6.96 - 8.85]	0.678
HbA1c whole (%)	8.08 [7.5 - 8.56]	7.86 [7.3 - 8.72]	0.625
Key: not normally distributed quantitative data: median [quartile1-quartile3];			

Table 3. Characterization of type 1 diabetes patients group and control group.

	Type 1 diabetes group	Control group	P-value
Number	52	37	-
Sex *Males* *Females*	 29 (55.8 %) 23 (44.2 %)	 14 (37.8 %) 23 (62.2 %)	0.095
Age (years)	28.5 [24.75 - 38.25]	34 [27 - 39]	0.161
BMI (kg/m^2)	23.17 [21.07 - 25.93]	23.62 [19.92 - 27.18]	0.662
Abdominal circumference	82 [75.75 - 97.25]	82 [73 - 92]	0.466
Hip circumference	96 [93 - 105]	100 [90 - 106]	0.963
Waist-hip ratio	0.86 [0.81- 0.90]	0.83 [0.77- 0.88]	0.607
Weight variation (last year) (kg)	0 [0 - 0]	0 [0 - 2]	0.689
Systolic blood pressure (mmHg)	125 [120 - 130]	120 [110 - 120]	< 0.001
Diastolic blood pressure (mmHg)	80 [70 - 85]	75 [60 - 80]	0.045
Increased blood pressure	11 (21.2 %)	6 (16.2 %)	0.559
Parental history of fracture	12 (23.08 %)	7 (18.92 %)	0.637
Personal history of fracture	1 (1.92 %)	0 (0 %)	1
Personal trauma history	10 (19.23 %)	9 (24.32 %)	0.563
Smoking status *Ex-smoker* *Current smoker* *Non-smoker*	 5 (9.6 %) 23 (44.2 %) 24 (46.2 %)	 6 (16.2 %) 14 (37.8 %) 17 (45.9 %)	0.614
Alcohol consumption *Denies* *Occasional* *Frequent*	 14 (26.92 %) 36 (69.23 %) 2 (3.85 %)	 12 (32.43 %) 25 (67.57 %) 0 (0 %)	0.436
Coffee consumption	44 (84.6 %)	31 (83.8%)	0.915
Physical activity *Vigorous* *Moderate* *Low* *Sedentary*	 6 (11.5 %) 25 (48.1%) 18 (34.6 %) 3 (5.8 %)	 1 (2.7 %) 21 (56.8 %) 14 (37.8 %) 1 (2.7 %)	0.395
Sun exposure *Frequent* *Moderate* *Low*	 3 (5.8 %) 27 (51.9 %) 22 (42.3 %)	 2 (5.4 %) 24 (64.9 %) 11 (29.7 %)	0.458
Milk products intake *Avoids* *Occasional* *Weekly* *Every day*	 3 (5.8 %) 8 (15.4 %) 23 (44.2 %) 18 (34.6 %)	 0 (0.0 %) 5 (13.5 %) 19 (51.4 %) 13 (35.1 %)	0.489
Calcium supplements	9 (17.3 %)	6 (16.2 %)	0.892
Magnesium supplements	12 (23.1 %)	8 (21.6 %)	0.871
Vitamin D supplements	2 (3.8 %)	4 (10.8 %)	0.196
Key: qualitative data: n (%), n=number of patients, %=percentage; not normally distributed quantitative data: median [quartile1-quartile3];			

Characterization of the healthy control group. Healthy control group included 14 men and 23 women. Median age in this group was 34 [27-39] years, median BMI 23.62 [19.92-27.18] kg/m^2. Characteristics of these participants, comparatively with those of the T1DM group, are depicted in Table 3. There was no statistically significant difference between the group with diabetes and control group in terms of age (p=0.161) and BMI (p=0.662).

Bone mineral density in patients and controls. There was no significant difference in

BMD between the group with T1DM and control group neither in lumbar spine (p=0.618), nor in femoral neck (p=0.974) or total hip (p=0.883). Extended data can be found in Table 4. Using a multiple linear regression model having BMD as dependent variable and BMI, age, sex and presence of T1DM as independent ones, we found no statistically significant association between presence of T1DM and BMD. We found that an increase in BMI is associated with an increase of BMD in all sites: lumbar spine (p=0.003), femoral neck (p=0.008) and total hip (p=0.003). An increase in age was associated with lower BMD at femoral neck (p=0.029) with no influence on BMD at lumbar spine and total hip.

Table 4. Bone mineral density in patients and controls.

		Type 1 diabetes group	Control group	P-value
ALL SUBJECTS	Number	52	37	-
	BMD lumbar spine L1-L4 (g/cm^2)	1.17 (± 0.14)	1.15 (± 0.13)	0.618
	BMD femoral neck (g/cm^2)	1.02 (± 0.13)	1.02 (± 0.14)	0.974
	BMD total hip (g/cm^2)	1.02 (± 0.14)	1.03 (± 0.14)	0.883
MALE SUBJECTS	Number	29	14	-
	BMD lumbar spine L1-L4 (g/cm^2)	1.15 (± 0.14)	1.15 (± 0.14)	0.893
	BMD femoral neck (g/cm^2)	1.01 (± 0.13)	1.06 (± 0.11)	0.288
	BMD total hip (g/cm^2)	1.03 (± 0.12)	1.10 (± 0.11)	0.095
FEMALE SUBJECTS	Number	23	23	-
	BMD lumbar spine L1-L4 (g/cm^2)	1.18 (± 0.12)	1.15 (± 0.12)	0.309
	BMD femoral neck (g/cm^2)	1.01 (± 0.12)	0.98 (± 0.14)	0.505
	BMD total hip (g/cm^2)	1.01 (± 0.15)	0.98 (± 0.12)	0.488
Key: normally distributed quantitative data: means (± SD);				

Table 5. Multiple linear regression assessing BMD in patients with type 1 diabetes.

	Lumbar spine L1-L4		Femoral neck		Total hip	
	β	p	β	p	β	p
BMI (increase of 1 kg/m^2)	0.0095	**0.043**	0.0072	0.108	0.0097	0.067
Smoking status (current/ex smoker=1, non smoker=0)	-0.1203	**0.0011**	-0.078	**0.022**	-0.085	**0.032**
Milk products intake (daily and weekly=1, occasional and avoids=0)	-0.1271	**0.0043**	-0.049	0.232	-0.04	0.395
Diabetes duration (increase of 1 year)	-0.0046	0.069	-0.0062	**0.012**	-0.0038	0.173
Presence of chronic complications=1	0.0531	0.229	0.066	0.118	0.042	0.389
Sex (females=1, males=0)	0.036	0.299	-0.019	0.555	-0.029	0.446
Age (increase of 1 year)	-8e-04	0.681	-0.0024	0.212	-9e-04	0.694
Statistic significance of the model	p = 0.001		p = 0.025		p = 0.164	
Key: β=regression coefficient;						

Factors influencing bone mineral density in the T1DM group. We analyzed the factors that could influence BMD in T1DM patients group using multiple linear regressions. Independent variables were considered BMI, sex, age, smoking status, milk products intake, diabetes duration and presence of T1DM chronic complications. We found a statistically significant positive association between BMI and BMD in lumbar spine (p=0.043). Statistically significant negative associations were found between smoking and BMD in all sites (p=0.001 for lumbar spine, p=0.022 for femoral neck, p=0.032 for total hip), between diabetes duration and BMD in femoral neck (p=0.012) and between milk products intake and

BMD at lumbar spine level (p=0.0043). Details are displayed in Table 5. On a separate analysis, no statistically significant associations were found between BMD and diabetes control (HbA1c), daily insulin dose, chronic diabetes complications (data not shown).

Discussions

In our study we compared BMD between a group of young T1DM patients, males and females, and a matched control group. We did not find a difference between BMD in diabetic patients and controls neither in lumbar spine nor in hip, data being analyzed taking the group as a whole and also separately on males and females. Additionally, we found no association between the presence of T1DM and BMD. Our results are consistent with published studies that included young adults. Thus, a study by Ingberg et al. (2004) on 38 patients with long-standing T1DM found no differences between BMD in diabetics and controls, in this study mean age and mean disease duration in patients were higher than in our study [13]. Hadjidakis et al. (2006) stated that premenopausal diabetic women lumbar spine BMD did not differ from controls but femoral neck BMD values in diabetic women were lower in controls and so were BMD values in male patients both at the lumbar spine and hip [15]. Hamilton et al. (2008), after adjustment for age and BMI, found only a difference for spine BMD between male patients and controls but no differences at other sites and in the female group [7]. Gogas et al. (2011) found that femoral and lumbar BMDs were lower in diabetics than in controls [32]. These studies suggest that T1DM may have an influence on trabecular and mixed cortical-trabecular bone, and larger studies are needed in order to sustain these findings.

We analyzed the factors that could influence BMD in T1DM group: BMI, sex, age, smoking status, milk products intake, diabetes duration and presence of complications. We found that BMI increase is associated with increased BMD in lumbar spine, while smoking is associated with lower BMD in all sites. Milk products intake considered qualitatively (patients having an intake daily or weekly were considered consumers, the others non-consumers) was negatively associated with BMD at lumbar spine level. This paradoxical association may be a result of either the subjective evaluation of milk consumption by questionnaire or of the limited number of subjects analyzed.

Regarding the factors specifically related to diabetes, we found an inverse significant association between diabetes duration and BMD in femoral neck. This relationship was also described by Ingberg et al. (2004), who observed that increased diabetes duration is associated with decreased lumbar BMD in men and femoral neck BMD in women [13]. Gogas et al. (2011) also found that diabetes duration is inversely correlated to femoral BMD [32]. Hadjidakis et al. (2006) on the other hand found no relationship between diabetes duration and BMD [15]. Our finding that longer diabetes duration lowers BMD in femoral neck may explain the higher incidence of hip fractures found in postmenopausal T1DM women [4].

No associations were present between BMD and diabetes control (HbA1c) or daily insulin dose. In our study, diabetes chronic complications (ischemic cardiac disease, cerebrovascular disease, retinopathy and neuropathy) were not associated with BMD values. Regarding these, results of other studies are contradictory [2,10,17-21].

Our study has several limitations. A limitation is the transversal (cross-sectional) design, our patients being evaluated only once in their disease evolution. These types of studies do not allow the identification of a causal relationship between BMI, smoking status, diabetes duration and risk of fractures. Another limitation is the use of questionnaires and not

direct evaluation of alcohol and milk dairy products consumption. The data obtained from the questionnaires are subjective and responses to questionnaires are influenced by the personality structure and emotional factors. Additionally, the number of patients enrolled is relatively small, this being another limitation of our study.

Conclusions

We did not identify differences between BMD in T1DM patients and controls in our study. However, studies on larger groups of diabetic patients should be carried on. The link between diabetes duration and BMD that we found, sustained by other studies, suggests that patients with long-standing T1DM should have their BMD screened.

REFERENCES

1. **Räkel A, Sheehy O, Rahme E, LeLorier J**. Osteoporosis among patients with type 1 and type 2 diabetes. *Diabetes Metab* 34: 193-205, 2008.

2. **Isidro ML, Ruano B**. Bone disease in diabetes. *Curr Diabetes Rev* 6: 144-155, 2010.

3. **Silva MJ, Brodt MD, Lynch MA et al**. Type 1 diabetes in young rats leads to progressive trabecular bone loss, cessation of cortical bone growth, and diminished whole bone strength and fatigue life. *J Bone Miner Res* 24: 1618-1627, 2009.

4. **Schneider S, Shapses SA**. Link Between Diabetes and Osteoporosis. *Medscape Diabetes & Endocrinology* 5, 2003, URL http://www.medscape.com/viewarticle/465736.

5. **Schwartz AV**. Diabetes Mellitus: Does it Affect Bone?. *Calcif Tissue Int* 73: 515-519, 2003.

6. **Massé PG, Pacifique MB, Tranchant CC et al**. Bone metabolic abnormalities associated with well-controlled type 1 diabetes (IDDM) in young adult women: a disease complication often ignored or neglected. *Nutr* 29: 419-429, 2010.

7. **Hamilton EJ, Rakic V, Davis WA et al**. Prevalence and predictors of osteopenia and osteoporosis in adults with Type 1 diabetes. *Diabet Med* 26: 45-52, 2009.

8. **Danielson KK, Elliott ME, LeCaire T, Binkley N, Palta M**. Poor glycemic control is associated with low BMD detected in premenopausal women with type 1 diabetes. *Osteoporos Int* 20: 923-933, 2009.

9. **Moyer-Mileur LJ, Slater H, Jordan KC, Murray MA**. IGF-1 and IGF-binding proteins and bone mass, geometry, and strength: relation to metabolic control in adolescent girls with type 1 diabetes. *J Bone Miner Res* 23: 1884-1891, 2008.

10. **Strotmeyer ES, Cauley JA, Orchard TJ, Steenkiste AR, Dorman JS**. Middle-aged premenopausal women with type 1 diabetes have lower bone mineral density and calcaneal quantitative ultrasound than nondiabetic women. *Diabetes Care* 29: 306-311, 2006.

11. **Rachoń D, Myśliwska J, Suchecka-Rachoń K, Semetkowska-Jurkiewicz B, Zorena K, Łysiak-Szydłowska W**. Serum interleukin-6 levels and bone mineral density at the femoral neck in post-menopausal women with Type 1 diabetes. *Diabet Med* 20: 475-480, 2003.

12. **Brandao FR, Vicente EJ, Daltro CH, Sacramento M, Moreira A, Adan L**. Bone metabolism is linked to disease duration and metabolic control in type 1 diabetes mellitus. *Diabetes Res Clin Pract* 78: 334-339, 2007.

13. **Ingberg CM, Palmér M, Aman J, Arvidsson B, Schvarcz E, Berne C**. Body composition and bone mineral density in long-standing type 1 diabetes. *J Intern Med* 255: 392-398, 2004.

14. **Valerio G, del Puente A, Esposito-del Puente A, Buono P, Mozzillo E, Franzese A**. The Lumbar bone mineral density is affected by long-term poor metabolic control in adolescents with type 1 diabetes mellitus. *Horm Res* 58: 266-272, 2002.

15. **Hadjidakis DJ, Raptis AE, Sfakianakis M, Mylonakis A, Raptis SA**. Bone mineral density of both genders in Type 1 diabetes according to bone composition. *J Diabetes Complications* 20: 302-307, 2006.

16. **Kassem HS, Arabi A, Zantout MS, Azar ST**. Negative effect of leptin on bone mass in type 1 diabetes. *Acta Diabetol* 45: 237-241, 2008.

17. **Miazgowski T, Pynka S, Noworyta-Zietara M, Krzyzanowska-Swiniarska B, Pikul R**. Bone mineral

density and hip structural analysis in type 1 diabetic men. *Eur J Endocrinol* 156: 123-127, 2007.

18. Salvatoni A, Mancassola G, Biasoli R et al. Bone mineral density in diabetic children and adolescents: a follow-up study. *Bone* 34: 900-904, 2004.

19. Aboelasrar MA, Elbarbary NS, Elshennawy DE, Omar AM. Insulin-like growth factor-1 cytokines cross-talk in type 1 diabetes mellitus: Relationship to microvascular complications and bone mineral density. *Cytokine* 59: 86-93, 2012.

20. Maser RE, Stabley JN, Lenhard MJ, Provost-Craig MA. Autonomic nerve fiber function and bone mineral density in individuals with type 1 diabetes: a cross-sectional study. *Diabetes Res Clin Pract* 84: 252-258, 2009.

21. Bridges MJ, Moochhala SH, Barbour J, Kelly CA. Influence of diabetes on peripheral bone mineral density in men: a controlled study. *Acta Diabetol* 42: 82-86, 2005.

22. Eller-Vainicher C, Zhukouskaya VV, Tolkachev YV et al. Low bone mineral density and its predictors in type 1 diabetic patients evaluated by the classic statistics and artificial neural network analysis. *Diabetes Care* 34: 2186-2191, 2011.

23. Hamann C, Kirschner S, Günther KP, Hofbauer LC. Bone, sweet bone--osteoporotic fractures in diabetes mellitus. *Nat Rev Endocrinol* 8: 297-305, 2012.

24. Pepene CE, Seck T, Diel I, Minne HW, Ziegler R, Pfeilschifter J. Influence of fluor salts, hormone replacement therapy and calcitonin on the concentration of insulin-like growth factor (IGF)-I, IGF-II and transforming growth factor-beta 1 in human iliac crest bone matrix from patients with primary osteoporosis. *Eur J Endocrinol* 150: 81-91, 2004.

25. Pepene CE, Seck T, Diel I, Minne HW, Ziegler R, Pfeilschifter J. Concentration of insulin-like growth factor (IGF)-I in iliac crest bone matrix in premenopausal women with idiopathic osteoporosis. *Exp Clin Endocrinol Diabetes* 112: 38-43, 2004.

26. Hie M, Iitsuka N, Otsuka T, Tsukamoto I. Insulin-dependent diabetes mellitus decreases osteoblastogenesis associated with the inhibition of Wnt signaling through increased expression of Sost and Dkk1 and inhibition of Akt activation. *Int J Mol Med* 28: 455-462, 2011.

27. Hofbauer LC, Hamann C, Ebeling PR. Approach to the patient with secondary osteoporosis. *Eur J Endocrinol* 162: 1009-1020, 2010.

28. Lichiardopol R , Florentiu A, Radoi V. Body composition and the metabolic impact of weight excess in patients with type 1 and type 2 diabetes mellitus. *Acta Endo (Buc)* 6: 493-506, 2010.

29. Graur M, Hancu N, Popa AR et al. Complicatii microvasculare. In: *Complicaţiile cronice ale diabetului zaharat*. Mezei AP (ed). Editura FarmaMedia, Târgu Mureş, pp 59-142, 2008.

30. Pepene CE. Evidence for visfatin as an independent predictor of endothelial dysfunction in polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 76: 119-125, 2012.

31. R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

32. Gogas Yavuz D, Keskin L, Kıyıcı S et al. Vitamin D receptor gene BsmI, FokI, ApaI, TaqI polymorphisms and bone mineral density in a group of Turkish type 1 diabetic patients. *Acta Diabetol* 48: 329-336, 2011.