PREVALENCE AND RISK FACTORS OF OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN WITH TYPE 2 DIABETES MELLITUS

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SUMMARY

Objective: Patients with type 2 diabetes (T2DM) are at increased risk of fractures. The aim of this study is to analyze the prevalence and risk factors of osteoporosis and osteoporosis related fractures in postmenopausal women with T2DM.

Methods: A total of 112 postmenopausal women with T2DM and 171 control nondiabetic women received a standardized questionnaire on osteoporosis risk factors, and were evaluated for bone mineral density (BMD, by using a dual energy X-ray absorptiometry), biochemical markers of bone and glucose metabolism, soluble receptor for advanced glycation end products (sRAGE) and its gene polymorphisms (rs1800625 or rs2070600).

Results: In T2DM patients the prevalence of osteoporosis was 25% and low trauma vertebral (Vfx) and non-vertebral fractures were found in 8% and 19% women, respectively. When compared between subjects with and without fractures, there were no significant differences in BMD at any site between the groups, except for distal radius, which was significantly lower in T2DM women with Vfx ($p < 0.05$ vs. non-fractured without osteoporosis). We found no associations between bone and glucose metabolism variables, sRAGE and BMD. No significant differences were observed in sRAGE levels according to their rs1800625, rs 2070600 genotype or fracture prevalence. Serum osteocalcin was significantly lower in T2DM women ($p < 0.01$ vs. controls) and in T2DM women with Vfx ($p < 0.05$) vs. non-fractured without osteoporosis. T2DM women with low daily walking activity (< 2 h daily) had significantly higher serum sclerostin levels ($p < 0.05$ vs. those who were walking > 2 h daily).

Conclusion: Diabetes-specific parameters as well as RAGE polymorphisms did not associate with BMD or fractures in T2DM postmenopausal women. Lower levels of osteocalcin, namely in those with Vfx and higher sclerostin levels in those with low daily walking activity suggest lower bone remodeling and/or decreased bone quality in T2DM.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) and osteoporosis are frequent metabolic disorders that belong to the most important causes of mortality and morbidity in elderly population. It has become apparent that patients with T2DM are at higher risk of fracture (1, 2) despite the evidence that T2DM patients present generally normal or increased bone mineral density (BMD) (3). Resulting from the presence of other diabetic complication, such as diabetic neuropathy or retinopathy or appearance of hypoglycemic episodes, the increased risk of fall is evident, especially in older adults with T2DM. Also other factors influence the risk of osteoporosis in T2DM patients including low calcium diet, vitamin D insufficiency, physical inactivity, smoking, and also genetic predisposition. However, the exact underlying mechanism leading to higher bone fragility inT2DM patients is not fully understood. Altered bone quality rather than bone mass reduction seems to play an essential role in the pathogenesis of bone fragility in these patients. The bone quality depends on the bone microarchitecture, bone remodelling and bone matrix properties that can be influenced by age, hyperglycemia and accumulation of advanced glycation end products (AGEs) within the bone or a loss of muscle mass and/or muscle strength (4). AGEs pathological effects are possibly suppressed by the soluble receptor for advanced glycation end products (sRAGE). Gene polymorphism of the receptor for advanced glycation end products (RAGE) was associated with serum circulating sRAGE (5). Studies regarding the bone metabolism have observed that low serum levels of sRAGE were associated with an increased risk of vertebral fractures (6) and accumulation of AGEs in the bone disturbs the bone remodelling (7). Differences in bone remodelling have been explored in T2DM (8, 9), several studies have reported lower serum osteocalcin levels in patients with T2DM than control subjects (10). In nondiabetic population, markers of bone remodelling are shown to predict fracture (11). Since BMD is not sufficient to predict fracture in T2DM population, other indicators of poor bone quality and higher bone fragility in T2DM patients need to be assessed. One of the promising markers is sclerostin that is produced by osteocytes, it acts as negative regulator of bone formation and mediates the bone response to mechanical

unloading through antagonizing Wnt/beta-catenin signaling (12). Circulating sclerostin levels found in T2DM patients were higher than in control subjects without T2DM (13).

The aim of our study was to analyze the prevalence and risk factors of osteoporosis and/or low trauma fractures, biochemical markers of bone remodelling and to investigate the role of sRAGE and its gene polymorphism in postmenopausal women with T2DM and control nondiabetic subjects.

MATERIALS AND METHODS

Our cross-sectional study comprised a convenience sample of postmenopausal women with T2DM and control subjects. From October 2011 to October 2013, we recruited postmenopausal women who attended a preventive bone mineral density (BMD) measurement. All the participants received a standardized questionnaire on osteoporosis risk factors regarding the fracture prevalence, physical activity, smoking and dairy products intake. The fracture related questions were detailed with respect to skeletal location, date of fracture occurrence (year) and trauma severity. Vertebral and typical nonvertebral fractures, namely hip, forearm, rib, tibia, humerus, and other fractures were analyzed; but not typical osteoporotic fractures, e.g. fractures of fingers were excluded. A fracture occurring spontaneously or after a fall from standing position was defined as a low trauma fracture. Fractures caused by traumatic events were excluded from the analysis. Furthermore, standardized questions on the history of diabetes were included in the questionnaire, including diabetic specific parameters (therapy, duration of diabetes). Patients suffering from other diseases than osteoporosis and T2DM that would interfere with bone metabolism such as primary hyperparathyroidism, liver disease, malabsorption, history of diabetic nephropathy, neuropathy or retinopathy were excluded from the study. Exclusion criteria included also abnormal serum calcium level, serum creatinine level >110 μmol/l or use of medication affecting bone metabolism within the 5 years prior to the selection, such as bisphosphonates, raloxifene, strontium ranelate, fluoride, glucocorticoids, thiazolidinedions, hormone replacement therapy, or active vitamin D supplements. A total of 112 postmenopausal women with T2DM (mean age 65.6 ± 9.4 years) met the inclusion criteria and fulfilled the questionnaire on osteoporosis risk factors.

The same exclusion criteria were respected also for the control group, that included 171 postmenopausal women without T2DM (mean age 64 ± 9.5 years). The study was undertaken with the understanding and written consent of each subject, with the approval of the Ethics Committee of the General University Hospital, and in compliance with the National Legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

Bone Mineral Density Measurement
BMD was measured using a dual energy X-ray absorptiometry (DXA) densitometer (Discovery A, Hologic, Inc, MA, USA, Software vision: Apex 3.0). BMD was measured at the lumbar spine (L1-L4), the total femur, the femoral neck and the distal radius. Osteoporosis was defined as T-score ≤ -2.5 SD, osteopenia as T-score between -1 and -2.5 SD and normal bone mineral density as T score ≥ -1 SD.

Laboratory Analysis
Routine biochemical analyses were performed with fresh samples; other aliquots were stored at -70°C before being analyzed. The serum calcium levels were measured by standard automated analytical procedure (Modular; Roche Diagnostics, Germany). The serum fasting glucose was measured by using the enzymatic colorimetric (GOD-PAP) method. The serum glycated hemoglobin (HbA1c) concentrations were assessed by high performance liquid chromatography. The serum concentration of C-terminal telopeptide of typeI collagen (βCTX), serum intact N-terminal propeptide of type procollagen (PINP), serum osteocalcin as well as serum total 25-hydroxyvitamin D (25-OH D) were measured using the electrochemiluminescence-based immunoanalysis (Modular; Roche Diagnostics, Germany). Fasting serum sRAGE levels concentrations were measured by the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) using standard kits Quantikine, RD Systems, Minneapolis, USA, according to the manufacturer's protocol. Serum sclerostin concentrations were measured using ELISA (Biomedica, Vienna, Austria).

Genotyping Analysis
Genomic DNA was isolated from blood using MagNA Pure Compact Nucleic Acid Isolation Kit 1, ROCHE,Germany. Assessment of allelic discrimination for the RAGE rs1800625 (assay IDs: C_8848033_1, rs2070600 (assay IDs: C_15867521_20), single nuclotide polymorphism was performed by the TaqMan assay with an Applied Biosystems Real-Time Polymerase Chain Reaction (PCR) System (Applied Biosystems, Foster City, CA), and further evaluated with Sequence Detection System version 3.0 software (Applied Biosystems). The total volume of TaqMan assays was 25 μL.

Statistical Analysis
Data were expressed by means and standard deviations if not otherwise stated. T test or one-way analysis of variance (ANOVA) with Tukey test for multiple comparison procedures was used to determine differences between groups. The associations were analyzed using multiple linear regression analysis. Due to the low prevalence of fragility fractures no multivariate analysis on fracture determinants was performed. χ^2 test was used to compare qualitative data. The genotype frequencies for each polymorphism were calculated in all groups. Hardy-Weinberg equilibrium (HWE) within the groups was estimated by χ^2 test. The significance was reached with a p value <0.05. Statistical analyses were made using the SigmaStat statistical software v.3.5 (Jandel, San Rafael, USA).

RESULTS

The characteristics of women enrolled in this study are stated in Table 1, the summary of completed questionnaire on osteoporosis risk factors and life style is shown in Table 2. We observed no

significant differences in self-reported history of smoking and dairy product consumption between T2DM women and control subjects (Table 2). T2DM women presented lower frequency of walking activity (the majority of T2DM women were walking less than 2 hours a day) than postmenopausal women without T2DM in the control group. Women with T2DM had higher BMI ($p < 0.001$) and higher prevalence of obesity (54%) than nondiabetic women (19%) (Table 1). The osteoporosis prevalence was lower in T2DM women (25%) than in control subjects (51%). 47% of T2DM women had osteopenia and 28% normal BMD, the prevalence in control subjects was 39% and 10%, respectively. Postmenopausal women with T2DM had higher BMD at the lumbar spine ($p < 0.001$), total femur ($p < 0.001$) as well as femoral neck ($p < 0.01$) than the control group. BMD at the distal radius showed no significant difference between groups (Table 1). We observed a significant and positive correlation between BMI and FN-BMD ($p < 0.05$).

Biochemical Analysis

Serum concentrations of osteocalcin were significantly lower in the T2DM women ($p < 0.01$ vs. controls). The marker of bone collagen synthesis serum PINP and bone resorption marker serum βCTX, were also lower than in controls, but the difference did not reach statistical significance (Table 1). We observed a significant and negative association between BMI and serum osteocalcin ($p = 0.001$), but not with serum PINP or βCTX (data not shown). We have found a significantly lower serum 25-hydroxyvitamin D level in T2DM women than in control subjects ($p < 0.001$). Serum concentrations of sRAGE and sclerostin did not significantly differ between T2DM women and control subjects (Table 1). In T2DM women, we did not find association between BMD and fasting glucose, HbA1c, duration of diabetes or serum sRAGE concentrations. Similarly, no association was observed between bone remodelling markers or sRAGE and fasting glucose or glycated hemoglobin.

Genotyping Analysis

When we analyzed the genotype frequencies of rs1800625 and rs2070600 in T2DM postmenopausal women and control subjects, we did not observe any significant differences (Table 3). Similarly, no significant differences were observed in serum sRAGE concentration according to their rs1800625 or rs2070600 genotype (Table 3). When we analyzed subject with and without history of low trauma fractures, we did not find any difference in analyzed RAGE genotype frequencies.

Fracture Analysis

Despite higher BMD and lower osteoporosis prevalence in T2DM women we found 8% prevalence of low trauma fractures of vertebrae, 13% of forearm, 0% of hip, and 6% of other nonvertebral low trauma fractures in T2DM women and in the control group 15%, 18%, 2% and 2%, respectively (Table 1). Occurrence of low trauma fracture was related to lower BMD values, however, most T2DM women with fractures had BMD values above a threshold of T-score of −2.5 SD.

When we compared groups of women with and without history of low trauma osteoporotic fractures we observed no significant difference in serum concentrations of bone remodelling markers as well as serum sclerostin or sRAGE concentrations in both diabetic women and nondiabetic control subjects (data not shown). When we subanalyzed women with prevalent vertebral fracture (Vfx), we observed significantly lower osteocalcin levels ($p < 0.01$) and trend to lower bone remodelling markers in T2DM women with Vfx when compared either with T2DM women with osteoporosis without Vfx or with nonfractured group without osteoporosis (Table 4). Serum concentrations of sclerostin and sRAGE did not significantly differ between these subgroups (Table 4). When we compared subgroups of control subjects with Vfx with non-Vfx subjects fulfilling the densitometric criteria for osteoporosis we did not find any significant difference in the concentrations of biochemical variables (Table 4). When we compared BMD in women with Vfx with those without osteoporosis or low trauma fractures, we found a significantly lower BMD at the distal radius in the Vfx group.

Antidiabetic Treatment

Majority of T2DM patients were treated with metformin (51%), 22% of patients were treated with combination of metformin and sulfonylurea derivates, gliptins or insuline, 4% of patients were treated by sulfonylurea derivates, and 4% by insulin. 19% of T2DM patients were on diabetes diet. We evaluated the biochemical parameters and BMD indices in patients treated with metformin in comparison with patients on diabetes diet. No significant differences were observed in biochemical indices or BMD between these subgroups of T2DM women.

Physical Activity and Bone Mineral Density

Using data from the questionnaire, we evaluated the effect of daily walking on bone metabolism. T2DM women walking less than 2 hours a day had significantly higher serum sclerostin concentrations (mean value 49.5 ± 17.9 pmol/l) than T2DM women walking more than 2 hours a day (mean value 41.1 ± 15.2 pmol/l) ($p < 0.05$) (Fig.1). We found no significant differences in lumbar spine, femoral neck or total femur BMD between T2DM subgroups. In the control group, postmenopausal women who were walking less than 2 hours a day had significantly lower
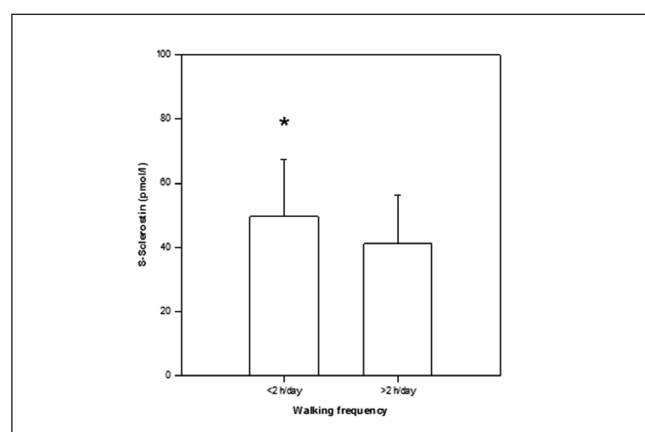

Fig. 1. Serum sclerostin concentrations according to daily walking frequency in T2DM postmenopausal women.

Table 1. Patients characteristics, biochemical indices, bone mineral density, and prevalence of low-trauma fractures in post-menopausal women with and without T2DM

	Postmenopausal T2DM Women N = 112	Controls Nondiabetics N = 171	p value (t-test)
Age (years)	65.6 ± 9.4	64.0 ± 9.5	ns
Years after menopause (years)	16.6 ± 11.2	15.1 ± 9.5	ns
Height (cm)	160.2 ± 6.8	162.0 ± 6.5	< 0.05
Weight (kg)	83.5 ± 21.4	70.3 ± 17.0	ns
BMI (kg/m^2)	32.5 ± 8.1	26.8 ± 6.1	< 0.001
BMI < 25 (kg/m^2)	14 (12 %)	78 (45 %)	
BMI 25–30 (kg/m^2)	38 (34 %)	61 (36 %)	
BMI > 30 (kg/m^2)	60 (54 %)	32 (19 %)	
Duration of T2DM (years)	7.1 ± 6.5	–	
S-calcium (mmol/l)	2.29 ± 0.11	2.29 ± 0.11	ns
S-creatinine (µmol/l)	70.5 ± 21.6	70.4 ± 17	ns
eGFR (ml/s/1,73m^2)	1.20 ± 0.23	1.22 ± 0.18	ns
S-fasting glucose (mmol/l)	7.22 ± 2.1	5.14 ± 0.5	< 0.001
S-HbA1c (mmol/mol)	52.9 ± 16.2	37.8 ± 3.9	< 0.001
S-βCTX (ng/l)	401.7 ± 189.1	446.7 ± 195.5	ns
S-PINP (ug/l)	43.12 ± 20	47.76 ± 20.1	ns
S-OC (ug/l)	19.39 ± 6.4	22.81 ± 7.6	< 0.01
S-sRAGE (ng/l)	1399.0 ± 624	1523.2 ± 613	ns
S-25 OH D (ng/ml)	20.8 ± 11.5	29.4 ± 11.8	< 0.001
S-sclerostin (pmol/l)	47.0 ± 17.5	47.1 ± 19.9	ns
BMD lumbar spine (g/cm^2)	0.95 ± 0.16	0.86 ± 0.13	< 0.001
BMD total femur (g/cm^2)	0.92 ± 0.14	0.83 ± 0.13	< 0.001
BMD femoral neck (g/cm^2)	0.72 ± 0.12	0.69 ± 0.12	< 0.01
BMD distal radius (g/cm^2)	0.61 ± 0.08	0.60 ± 0.08	ns
No. of patients with forearm fx	14 (13%)	33 (18%)	
No. of patients with hip fx	0 (0%)	3 (2%)	
No. of patients with vertebral fx	9 (8%)	23 (15%)	
No. of patients with other low trauma fx.	8 (6%)	3(2%)	

BMI – Body Mass Index, eGFR – estimated Glomerular Filtration Rate, HbA1c – glycated hemoglobin A1c, βCTX – C-terminal telopeptide of type I collagen, PINP – N-terminal propeptide of type procollagen, OC – osteocalcin, sRAGE – soluble receptor for advanced glycation endproducts, 25OH D – 25-hydroxyvitamin D, BMD – Bone Mineral Density, T2DM – Type 2 Diabetes Mellitus, fx – fracture
Data are expressed as numbers (percentage) or Mean ± SD, ns – not significant.

lumbar spine and total femur BMD than women walking more than 2 hours a day.

DISCUSSION

There is substantial evidence that osteoporosis and related fractures is a clinically significant and commonly underestimated problem in patients with T2DM (1, 14). In our cohort of post-menopausal women with T2DM the prevalence of osteoporosis (defined by BMD) was 25% and the prevalence of low trauma vertebral and non-vertebral fractures was 8% and 19%, respectively. These results are in accordance with previously published studies; however, the prevalence of low trauma fractures varies according to the studied population (15, 16). In a study by Leidig-Bruckner et al., the reported prevalence of osteoporosis in women with T2DM was 21.9% and overall prevalence of low trauma fractures 5.7% (15). In another study the prevalence of osteoporosis in postmenopausal T2DM women was 30.4% at lumbar spine (LS) and 9.5% at femoral neck (FN) with 23% prevalence of vertebral fractures (16).

In agreement with others (3), we found a significantly higher BMD (at lumbar spine, total femur and femoral neck), except for radius in women with T2DM in comparison with a non-diabetic control group (Table 1). The increased BMD may be partly attributed to the higher BMI in T2DM women (Table 1). BMI is one of the major determinants of bone mass, and was positively correlated with BMD in postmenopausal women both with and without T2DM. This is in accordance with previous studies in nondiabetic postmenopausal women (17). The higher BMD in

overweight/obese subjects is presumed to result from a combination of the positive effects of increased mechanical loading on bone and increased estradiol levels due to increased conversion of androgen precursors to estrogen in the expanded adipose tissue volume. However, recent data from epidemiological studies suggested that although the prevalence of fractures is reduced as BMI goes from underweight to normal, an increase in BMI from overweight to obese is actually associated with a higher prevalence of fractures (18).

When compared between subjects with and without fractures, there were no significant differences in BMD at any site between both groups – T2DM and controls. The occurrence of low trauma fracture was related to lower BMD values, however, most T2DM women with fractures had BMD values above a threshold of T score of −2.5 SD. These data suggest that the higher areal BMD in obese diabetics does not result in biomechanical advantage. However, when we compared BMD in women with prevalent vertebral fracture (Vfx) with those without osteoporosis or low-trauma fractures, we found a significantly lower BMD only at the distal 1/3 radius in the Vfx group (Table 4). These results suggest that BMD at distal 1/3 radius, which evaluates predominantly cortical bone, may be the best site in T2DM patients to assess fracture risk. Although the areal BMD cannot distinguish between cortical and trabecular bone, our results are in accordance with recent studies, which reported increased cortical porosity at the radius and tibia in female diabetics who have fractured, as measured by intracortical pore volume fraction via high-resolution peripheral quantitative computed tomography (19). Also, in more recent community-based study (women and men were included), T2DM was associated with deficits in cortical microstructure and density at the distal tibia (20). Nevertheless, although BMD at distal radius in women with fractures was lower compared with the control group, BMD values were above a threshold of T-score of −2.5 SD suggesting that areal BMD are unable to discriminate T2DM patients with prevalent VFx from those without VFx. One possible explanation could be the presence of vitamin D deficiency and secondary hyperparathyroidism in T2DM women, as PTH causes predominantly cortical bone loss. It seems necessary to seek other imaging modalities or biochemical markers evaluating bone fragility and fracture risk in T2DM.

Our findings showed significant differences in serum osteocalcin levels between T2DM patients and control subjects. Moreover, in this study, serum osteocalcin was significantly lower in T2DM women with vertebral fractures than in those without osteoporosis and fractures (Table 4). It is well known that osteocalcin plays

Table 2. *Summary of results from a questionnaire on osteoporosis risk factors and life style in postmenopausal women with T2DM and control nondiabetic group*

	T2DM women N = 112	Control subjects N = 171
Current smoking		
No	91 (81%)	149 (87%)
Yes	21 (19%)	22 (13%)
Milk		
None	15 (13%)	31 (18%)
< 1/4 liter/day	66 (59%)	82 (48%)
< 1/2 liter/day	31 (28%)	50 (29%)
More	0 (0%)	8 (5%)
Dairy products		
None	0 (0%)	5 (3%)
Sometimes	6 (5%)	10 (6%)
Many times/week	37 (33%)	47 (27%)
Every day	69 (62%)	109 (64%)
Exercise		
Never	24 (22%)	36 (21%)
< 1 h/week	44 (39%)	52 (30%)
1–2 h/week	38 (34%)	59 (35%)
> 2 h/week	6 (5%)	24 (14%)
Walking		
None	0 (0%)	0 (0%)
Only with assistance	2 (2%)	3 (2%)
< 2 h/day	72 (64%)	79 (46%)
> 2 h/day	38 (34%)	89 (52%)

Table 3. *Comparison of serum sRAGE concentrations in T2DM postmenopausal women and control subjects in respect to their RAGE genotype (rs1800625, rs2070600) prevalence*

	T2DM women	Controls	X^2 (p value)	T2DM women	Controls
Genotypes	rs1800625 RAGE (T > C)			sRAGE (ng/l)	
TT	80 (71%)	112 (65%)	ns	1404.1 ± 578	1605.5 ± 548
TC	28 (25%)	54 (32%)	ns	1421.1 ± 779	1383.2 ± 694
CC	4 (4%)	5 (3%)	ns	1148.2 ± 522	2371.5 ± 1104
P value				ns	ns
Genotypes	rs2070600 RAGE (G > A)			sRAGE (ng/l)	
GG	99 (88%)	161 (94%)	ns	1436.4 ± 643	1538.2 ± 620
GA	13 (12%)	10 (6%)	ns	1151.0 ± 431	1249.2 ± 411
AA	0 (0%)	0 (0%)	ns	–	–
P value				ns	ns

Data for genotypes are number of patients (percentage), and Mean ± SD for sRAGE, ns – not significant
T2DM – Type 2 Diabetes Mellitus, RAGE – receptor for advanced glycation endproducts, sRAGE – soluble receptor for advanced glycation end products

an important role in bone metabolism. Recent studies suggest a wider dimension of osteocalcin function that extends from the bone metabolism to the regulation of energy metabolism. Undercarboxylated osteocalcin was found to act as a hormone that improves glucose metabolism. In animal studies, osteocalcin knocked out mice had higher fat mass, high serum glucose and triglyceride levels as well as decreased secretion of insulin and adiponectin (21). In agreement with previously published studies on T2DM subjects (22) or on obese nondiabetic women in comparison with non-obese women (23), our data in postmenopausal women with T2DM showed a significant negative relationship between osteocalcin and BMI. Although obesity is associated with low bone turnover, the relationship between serum osteocalcin and adiposity needs a full explanation. More recently, Cohen et al. (24) demonstrated that the higher trunk fat in obese healthy premenopausal women is associated with lower bone formation and inferior bone quality, as assessed by bone histomorphometry; however, the mechanism(s) which underline the aetiology for the reduced bone quality is still unknown.

Serum osteocalcin correlates with osteoblast number and bone formation rate as well as with the rate of new bone formation and old bone resorption. While a majority of studies showed a lower concentration of serum osteocalcin in T2DM patients than in control subjects (8, 9), the available reports on another biochemical bone turnover markers showed inconsistent results (8, 9). In our study, we found a significant positive correlation between serum osteocalcin and PINP or bone resorption marker CTX (data not shown). The marker of bone collagen synthesis serum PINP and bone resorption markers, serum βCTX and TRAP5b, were also lower (although differences did not reach statistical significance), suggesting a decrease in bone remodelling. There is a considerable heterogeneity in methodology and markers used to determine the bone resorption or formation. The study with obese postmenopausal Saudi women reported higher bone resorption markers in T2DM women then control subjects (25). Other studies have shown either no differences in CTX levels between diabetic male and control subjects (26), or decreased CTX levels in elderly women with T2DM compared to control subjects (27). The meta-analysis by Starup-Linde et al. that included 22 studies concerning the bone turnover in T2DM patients have reported lower β-CTX and osteocalcin concentration, however, PINP did not differ between T2DM patients and control group (9).

Decreased bone formation might occur in part because of increased AGEs in bone collagen. In the setting of chronic hyper-

Table 4. Comparison of bone mineral density and biochemical indices between postmenopausal women with vertebral fracture and with osteoporosis without vertebral fracture and control subjects both in T2DM and nondiabetics control group

	T2DM			Nondiabetics subjects		
	Verterbral Fx N = 9	Osteoporosis without Vfx N = 25	Osteopenia/ normal without fx N = 60	Verterbral Fx N = 26	Osteoporosis without Vfx N = 73	Osteopenia/ normal without fx N = 45
Age (years)	68.1 ± 4.3	72.1 ± 7.9*	62.2 ± 9.1	71.2 ± 7.3#•	64.8 ± 7.9*	58.9 ± 9,5
Height (cm)	157.8 ± 5.8	156.6 ± 6.5*	162.0 ± 6.3	159.2 ± 6.0#	161.4 ± 6.8	164.0 ± 6.3
Weight (kg)	81.3 ± 17.9	74.6 ± 16.9*	89.3 ± 23.7	66.7 ± 12.5	68.3 ± 11.7	74.8 ± 26.7
BMI (kg/m²)	32.8 ± 7.9	30.3 ± 5.6	34.1 ± 9.5	26.3 ± 4.7	26.3 ± 4.7	27.9 ± 9.2
Duration of T2DM (years)	10.1 ± 7.9	5.0 ± 4.1	7.3 ± 6.5	–	–	
S-calcium (mmol/l)	2.27 ± 0.07	2.35 ± 0.13	2.28 ± 0.11	2.25 ± 0.11	2.33 ± 0.11	2.29 ± 0.12
S-creatinine (μmol/l)	72.1 ± 15.5	73.3 ± 17.1	69.8 ± 27.0	71.6 ± 10.8	72.2 ± 23.2	68.9 ± 14.5
eGFR (ml/s/1,73 m²)	1.13 ± 0.18	1.09 ± 0.23	1.24 ± 0.23	1.12 ± 0.14	1.13 ± 0.17	1.23 ± 0.15
S-fasting glucose (mmol/l)	8.0 ± 1.8	7.2 ± 1.2	7.0 ± 2.4	5.1 ± 0.6	5.2 ± 0.5	5.1 ± 0.5
S-HbA1c (mmol/mol)	60.0 ± 20.6	48.3 ± 5.5	53.5 ± 19.9	38.5 ± 2.9	38.1 ± 4.3	37.3 ± 3.6
S-CTX (ng/l)	346.7 ± 134	421.1 ± 159	422.4 ± 207	415.0 ± 178	470.4 ± 218	446.5 ± 144
S-PINP (ug/l)	36.1 ± 13.6	41.8 ± 13.8	46.2 ± 25.4	47.3 ± 16.6	49.8 ± 23.7	45.5 ± 16.0
S-OC (ug/l)	16.2 ± 4.8#	22.0 ± 6.6	21.8 ± 4.9	21.1 ± 5.7	24.5 ± 8.6	22.0 ± 7.4
S-sRAGE (ng/l)	1416.4 ± 947	1433.8 ± 728	1353.8 ± 520	1460.5 ± 393	1544.2 ± 596	1517.0 ± 778
S-sclerostin (pmol/l)	52.1 ± 18.3	45.0 ± 19.3	46.1 ± 16.5	43.9 ± 18.4	47.1 ± 19.7	46.1 ± 18.8
BMD lumbar spine (g/cm²)	0.96 ± 0.19•	0.81 ± 0.10*	0.994 ± 0.141	0.82 ± 0.01#	0.79 ± 0.09*	0.956 ± 0.139
BMD total femur (g/cm²)	0.86 ± 0.12•	0.82 ± 0.11*	0.974 ±0.143	0.79 ± 0.12#	0.79 ± 0.08*	0.919 ± 0.171
BMD femoral neck (g/cm²)	0.69 ± 0.06	0.62 ± 0.08*	0.773 ± 0.124	0.66 ± 0.1#	0.66 ± 0.14*	0.738 ± 0.102
BMD distal radius (g/cm²)	0.59 ± 0.07#•	0.52 ± 0.07*	0.656 ± 0.055	0.56 ± 0.08#	0.57 ± 0.07*	0.651 ± 0.065

Data are expressed by means and standard deviations if not otherwise stated (Mean ± SD). One-way analysis of variance (ANOVA) with Tukey test for multiple comparison procedures was used to determine differences between groups. #p < 0.05 Vfx vs. osteopenia/normal without fx; •p < 0.05 Vfx vs. osteoporosis without Vfx; *p < 0.05 osteoporosis without Vfx vs. osteopenia/normal without fx

BMI – Body Mass Index, eGFR – estimated Glomerular Filtration Rate, HbA1c – glycated hemoglobin, A1c, βCTX – C-terminal telopeptide of type I collagen, PINP – N-terminal propeptide of type procollagen, OC – osteocalcin, sRAGE – soluble receptor for advanced glycation endproducts, BMD – Bone Mineral Density, T2DM – Type 2 Diabetes Mellitus, fx – fracture, Vfx – vertebral fracture

glycemia or AGEs, AGEs accumulate in the organic bone matrix and interfere with normal osteoblast function as well as impair osteoblast development (28). Interaction of AGEs with their cellular receptors (RAGE) has an important role in the pathogenesis of diabetic complications (29). Recent evidence support the hypothesis, that link between diabetes and osteoporosis may be mediated through accumulation of AGEs in the bone collagen that may affect the bone remodelling (30) and induce a deterioration of bone quality. In our study, no differences were observed in serum concentration of sRAGE between diabetic and nondiabetic women and no relationship of serum sRAGE concentrations with BMD, fracture prevalence or bone remodelling markers in T2DM women was found. These negative results may be partly explained by good glycemic control in our patients that could improve plasma sRAGE abnormalities and provide benefit to diabetic patients (31).

In T2DM patients (both men and women), higher sclerostin levels were associated with an increased risk of vertebral fractures independent of lumbar spine BMD (32). In our study we have found no differences in plasma sclerostin level between T2DM women and nondiabetic subjects, which is in contrast with some previously published studies that reported a significantly higher plasma sclerostin levels in T2DM patients than control subjects (13). However, in our cohort of T2DM postmenopausal women, we observed a significantly higher serum sclerostin levels in those walking for less than 2 hours daily (Fig. 1). These data suggests that discrepancies between sclerostin levels in T2DM studies reflect in fact the differences in physical activity. In our previous study we have found that limited walking ability contributes to a loss of BMD in the proximal femur in both premenopausal and postmenopausal women with multiple sclerosis (33). In the present study, we did not find any effect of walking frequency on lumbar spine, femoral neck or total femur BMD in T2DM postmenopausal women. In the control group postmenopausal women without T2DM with decreased walking activity (for less than 2 hours daily) had significantly lower lumbar spine and total femur BMD than women walking more than 2 hours a day. We suggest that the lack of relationship between walking activity and BMD in our study may be because the expanded adipose tissue volume and the fat distribution in soft tissues around the bone and within the marrow may affect DXA BMD measurements, namely in obese T2DM women. Sclerostin, an osteocyte product, a negative mediator of bone formation, mediates the bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling (12). Although, in our study population of postmenopausal women with or without T2DM no significant association between serum sclerostin levels and bone turnover markers was found, it was well documented that even minor changes in physical activity in healthy premenopausal women are associated with effects on serum levels of sclerostin and bone turnover markers (32). Moreover, in non-diabetic postmenopausal women, it was shown that already moderate level of physical activity, including walking, is associated with substantially lower risk of hip fracture in non-diabetic postmenopausal women (34). In older women with diabetes mellitus, difficulties with moderate physical activities, such as bending or stooping, or walking several blocks were significantly associated with incident fracture (35). Collectively, the studies conducted so far suggest that sclerostin could be a link between mechanical loading and disuse osteoporosis in humans and even minor changes in physical activity may influence bone quality and/or fracture risk. Further, namely longitudinal studies in diabetes are warranted.

Limitation of the Study

The cohort of patients and control subjects were recruited from the Centre of Metabolic Bone Diseases where they attended bone densitometric measurement and so the prevalence of osteoporosis in our control group could differ from the prevalence of osteoporosis in general population. Good glycemic control of T2DM patients in this study could influence the negative results in the metabolism of AGEs in our population. Due to cross-sectional design of our study we could not validly verify the effect of metformin treatment on bone metabolism before and during the treatment in each individual. Moreover, other nonskeletal parameters that are important determinants of fractures in addition to BMD, such as the risk of falls, muscle weakness, dizziness, and cerebrovascular diseases were not assessed within the present study. Also, the type of therapy (diet, oral antidiabetics) was not related to the risk of osteoporosis in women. None of our patients was treated with thiazolidinedione, so this known risk factor for osteoporosis was not relevant in our analysis. Finally, the relatively low number of patients with fragility fractures, namely Vfx in T2DM patients, limits conclusive statistical analysis, especially regarding fracture occurrence.

In conclusion, our results show that BMD are unable to discriminate T2DM patients with prevalent low trauma fractures from those without fractures. Diabetes-specific parameters as well as RAGE polymorphism did not predict BMD in T2DM postmenopausal women. Low levels of osteocalcin in T2DM, namely in those with vertebral fractures, suggest low bone remodelling and/or decreased bone quality. Moreover, our results showed that low walking activity in T2DM women is associated with a higher circulating sclerostin levels and with reduced BMD in control group. Daily walking activity can be introduced in a primary healthcare setting as a low-cost mode of exercise that promotes weight loss and improves not only fat and glucose metabolism but also bone health in T2DM patients. Further research, especially longitudinal studies, is needed to identify the clinically important risk factors that could explain impaired bone quality in T2DM population.

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