In Postmenopausal Female Subjects With Type 2 Diabetes Mellitus, Vertebral Fractures Are Independently Associated With Cortisol Secretion and Sensitivity

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Context: In type 2 diabetes (T2D), the vertebral fracture (VFx) prevalence and cortisol secretion are increased.

Objective: The objective of this study was to evaluate the role of glucocorticoid secretion and sensitivity in T2D-related osteoporosis.

Design and Setting: This was a case-control study in an outpatient setting.

Patients: The patients were ninety-nine well-compensated T2D postmenopausal women (age, 65.7 ± 7.3 y) and 107 controls (age, 64.5 ± 8.2 y).

Main Outcome Measures: We assessed osteocalcin, C-terminal telopeptide of type I collagen, ACTH, cortisol after the dexamethasone suppression test (F-1mgDST), *Bcl*I and N363S single-nucleotide polymorphisms (SNPs) of glucocorticoid receptor, lumbar spine and femoral neck bone mineral density by dual x-ray absorptiometry, and VFx by radiography.

Results: Compared with controls, T2D subjects had increased VFx prevalence (20 vs 34.3%, respectively; $P = .031$), bone mineral density (Z-scores, lumbar spine, 0.16 ± 1.28 vs 0.78 ± 1.43, $P = .001$; femoral neck, −0.03 ± 0.87 vs 0.32 ± 0.98, $P = .008$, respectively), and F-1mgDST (1.06 ± 0.42 vs 1.21 ± 0.44 μg/dL, 29.2 ± 1.2 vs 33.3 ± 1.2 nmol/L, respectively; $P = .01$), and decreased osteocalcin (10.6 ± 6.4 vs 4.9 ± 3.2 ng/mL, 10.6 ± 6.4 vs 4.9 ± 3.2 μg/L, respectively; $P < .0001$) and C-terminal telopeptide of type I collagen (0.28 ± 0.12 vs 0.14 ± 0.08 ng/mL, 0.28 ± 0.12 vs 0.14 ± 0.08 mcg/L, respectively; $P < .0001$). Fractured controls or T2D patients had increased sensitizing N363S SNP prevalence (20 and 17.6%, respectively) compared to non-fractured subjects (3.4 and 3.1%, respectively; $P = .02$ for both comparisons), and similar *Bcl*I SNP prevalence. The VFx presence was associated with the sensitizing variant of N363S SNPs in controls (odds ratio [OR] = 10.6; 95% confidence interval [CI], 1.8–63.3; $P = .01$) and in T2D patients (OR = 12.5; 95% CI, 1.8–88.7; $P = .01$), and with the F-1mgDST levels (OR = 2.1; 95% CI, 1.1–4.1; $P = .03$) only in T2D patients.

Conclusions: In postmenopausal T2D women, VFx are associated with cortisol secretion and the sensitizing variant of N363S SNPs. **(*J Clin Endocrinol Metab* 100: 1417–1425, 2015)**

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Abbreviations: BMD, bone mineral density; BMI, body mass index; β-CTX, C-terminal telopeptide of type I collagen; F-1mgDST, cortisol level after 1 mg overnight dexamethasone suppression test; FN, femoral neck; GC, glucocorticoid; GR, GC receptor; HbA1c, glycosylated hemoglobin; 11HSD, 11βhydroxysteroid dehydrogenase; 11HSD1, 11HSD type 1; LS, lumbar spine; OC, osteocalcin; ROC, receiver operating characteristic; R-UFF/UFE, UFF/UFE ratio; T2D, type 2 diabetes; UFE, urinary free cortisone; UFF, urinary free cortisol; VFx, vertebral fracture(s).

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Several studies suggest that patients with type 2 diabetes (T2D) have normal or even increased bone mineral density (BMD), but reduced bone quality and an increased risk of fracture (1–9). These discrepancies between BMD and the risk of fracture explain the limited role of dual-energy x-ray absorptiometry for assessing fracture risk in T2D patients (9). Osteoporosis in T2D is characterized by a low bone turnover with a normal/decreased bone resorption and decreased bone apposition, partially due to the inhibition of the Wnt/β-catenin signaling (10–12). Other mechanisms include the toxic effect on collagen of advanced glycosylation agents, caused by chronic exposure to glucose excess (13, 14). Indeed fracture risk is maximal in patients with T2D chronic complications, which are caused by chronic hyperglycemia (9, 15, 16).

Recently, cortisol secretion and sensitivity have been suggested to play a role in the pathogenesis of postmenopausal osteoporosis (17, 18). Indeed, in osteoporotic patients, the degree of cortisol secretion, even if in the normal range, seems to be associated with BMD (19–20), and the genetic variations of 11β-hydroxysteroid dehydrogenase (11HSD) activity seem to influence the severity of osteoporosis (21–23). Moreover, the different glucocorticoid (GC) receptor (GR) polymorphisms are thought to be associated with fracture risk in osteoporotic patients (24–27).

Also in T2D patients, cortisol secretion and/or sensitivity may influence the disease. Indeed, increased cortisol secretion seems to be present in T2D patients (28, 29), and the different GR polymorphisms may influence the diabetic control (30, 31).

Notwithstanding the potential role of cortisol secretion and sensitivity in the pathogenesis of postmenopausal osteoporosis and in influencing metabolic control in T2D, no studies have investigated cortisol secretion and sensitivity in T2D-related osteoporosis so far.

Therefore, the aim of the present study was to evaluate bone involvement in a group of diabetic postmenopausal female subjects with and without T2D, in relation to cortisol secretion and sensitivity.

Patients and Methods

Patients

From September 2011 to September 2014, 120 consecutive Caucasian subjects with T2D referred to our outpatient clinic for diabetes and 120 Caucasian nondiabetic subjects were enrolled in the study, which was approved by the local Ethical Committee.

T2D patients were selected on the basis of the following criteria: age range 50–85 years, postmenopausal status, age at T2D diagnosis > 30 years, BMI of 19–40 kg/m^2, no insulin therapy during the first 2 years of the disease, and glycosylated hemoglobin (HbA1c) ≤ 8.0%. The exclusion criteria were: signs of hypercortisolism (moon facies, striae rubrae, hypertrichosis, skin atrophy, and buffalo hump); history of ketoacidosis or hypoglycemia in the 6 months before enrollment; past or present therapy with glitazones, glucocorticoids (>3 mo and/or > 5 mg/d of prednisone equivalents), antidepressants, bisphosphonates, strontium ranelate, PTH (1–34 and 1–84), denosumab, anticonvulsants, estrogens, or selective estrogen receptor modulators; presence of hyperthyroidism, rheumatoid arthritis, scleroderma, malabsorption, neoplasia, hyperandrogenism, alcoholism, depression, chronic renal failure, acute illness, alterations of sleep-wake cycle; presence of proliferative or laser-treated retinopathy, overt diabetic nephropathy (macroalbuminuria > 300 mg/24 h), severe macroangiopathy (history of myocardial infarction, coronary artery bypass graft surgery, percutaneous transluminal coronary, and carotid, femoral, or femoral-popliteal angioplasty).

The nondiabetic subjects were consecutively recruited on the basis of the same inclusion and exclusion criteria from our outpatient clinic for endocrine diseases, where they were followed up for nonmorbid obesity, euthyroid nodular goiter, or euthyroid chronic lymphocytic thyroiditis.

Twenty-one diabetic and 13 nondiabetic subjects did not complete the study protocol and were excluded from the analysis, which has eventually been performed on 99 subjects with T2D and 107 subjects without T2D who served as controls.

Methods

In all subjects, a personal and familial history and two validated questionnaires regarding the propensity to fall (32) and daily calcium intake (33) were collected.

Venous blood samples were taken in the morning after a 10-hour fast. All blood and urine samples were immediately stored at −20°C until analysis. Total serum calcium corrected for albumin [total serum calcium + (4.4 − albumin mg/dL) × 0.8], 25-OH vitamin D, HbA1c, 24-hour urinary calcium excretion, and creatinine clearance were measured in all patients, and albumin excretion rate was measured in T2D subjects. Serum osteocalcin (OC) and C-terminal telopeptide of type I collagen (β-CTX) were measured in 48 subjects with T2D and 48 subjects without T2D. OC (normal values, 5–25 ng/mL) was measured by the Invitrogen human Osteocalcin Enzyme Amplified Sensitivity Immunoassay (Life Technologies) following the manufacturer's instructions, and β-CTX (normal values, 0.14–1.35 ng/mL) was determined by the Serum CrossLaps ELISA (Immunodiagnostic System Ltd) according to the manufacturer's assay procedure.

Cortisol secretion was evaluated by measuring morning plasma ACTH (normal values, 10–55 pg/mL, 2.2–12.1 pmol/L), 24-hour urinary free cortisol (UFF; normal values, 3–43 μg/24 h, 8.3–118.7 nmol/24 h), 24-hour urinary free cortisone (UFE; normal values, 15–122 μg/24 h, 41.6–337.9 mol/24 h), and serum cortisol at 9 AM after the administration of 1 mg dexamethasone at 11 PM the previous day (F-1mgDST; normal values, <1.8 μg/dL, 50 nmol/L).

At study entry, plasma morning ACTH levels (mean of three determinations at 20-min intervals) and serum cortisol levels (after dichloromethane extraction) were measured by immunoradiometric assay (BRAHMS Diagnostica GmbH) and immunofluorometric assay (TDX-FLX kits; Abbott Diagnostika GmbH). The intra- and interassay coefficients of variation were ≤ 10% for ACTH and < 3% for cortisol.

The UFF/UFE ratio (R-UFF/UFE) was used as a measure of the 11HSD type 2 activity. This enzyme, by inactivating cortisol to

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cortisone, may modulate the tissue exposure to active GC (22). The determination of UFF and UFE was performed by liquid chromatography-tandem mass spectrometry, after purification by an on-line TurboFlow system using a Cyclone column (Thermo Scientific). Cortisol and cortisone were separated in liquid chromatography (Hypersil Gold; Thermo Scientific). Detection and quantification were performed by a triple quadrupole mass spectrometer (TSQ Quantum Access; Thermo Scientific). The coefficient of variation is < 10%, the accuracy is between 98 and 100%, and the limit of quantification is 1 μg/L for both cortisol and cortisone (34).

Genomic DNA was isolated from peripheral blood leukocytes using an Illustra DNA Extraction Kit BACC2 (GE Healthcare). Detection of the *Bcl*I and N363S polymorphisms was assessed by PCR, as previously described (26).

In T2D subjects, the presence of chronic complications was evaluated. The diagnosis of diabetic neuropathy was based on symptoms and quantitative sensory and motor testing. Diabetic retinopathy was searched with funduscopic examination and categorized as nonproliferative, preproliferative, and proliferative. Diabetic nephropathy was assessed by measuring microalbumin in 24-hour urine (normal value, <30 mg/d) twice (at enrollment and after 3–6 mo) to determine persistent microalbuminuria (albumin excretion rate between 30 and 300 mg/d).

In all subjects, BMD was measured by dual-energy x-ray absorptiometry (Hologic Discovery) at the lumbar spine (LS; precision 1.0%) and femoral neck (FN; precision 1.8%) and expressed as Z-score and T-score (number of standard deviations above/below the mean for the patient's age, sex, and ethnicity, or above/below the mean for a healthy 30-year-old adult of the same sex and ethnicity as the patient, respectively).

Conventional spinal radiographs in lateral (T4–L4) and anteroposterior projection were obtained in all subjects with standardized technique. Two trained radiologists, blinded to BMD and hormonal data, independently reviewed the radiographs. Vertebral fractures (VFx) were diagnosed on visual inspection using semiquantitative visual assessment and defined as reductions of more than 20% in anterior, middle, or posterior vertebral height (35).

Statistical analysis

Statistical analysis was performed by SPSS version 21.0 statistical package (SPSS Inc).

The results were expressed as mean ± SD. The normality of distribution was tested by Kolmogorov-Smirnov test. The comparison of continuous variables between T2D and controls was performed using Student's *t* test or Mann-Whitney *U* test, as appropriate, and between T2D and controls with and without VFx by one-way ANOVA and Bonferroni post hoc analysis. Categorical variables were compared by χ^2 test or Fisher's exact test, as appropriate. The associations between the bone metabolism parameters, HbA1c, age, BMI, LS-BMD, FN-BMD, and cortisol secretion parameters were tested by the Pearson product-moment correlation or Spearman's correlation as appropriate. We tried to fit a relationship between OC and HbA1c and between OC and F-1mgDST into the whole sample of subjects and in both diabetic patients and nondiabetic subjects.

Patients were divided into tertiles of F-1mgDST or HbA1c for assessing the association between VFx prevalence and cortisol secretion or glycometabolic control, respectively. Logistic regression analysis assessed the association between VFx presence (as a dependent variable) and parameters known as risk factors (as independent variables), even considering the results of the comparisons of the clinical, biochemical, and genetic characteristics between subjects with and without T2D and with and without VFx. The receiver operating characteristic (ROC) curve analysis assessed the cutoff(s) of the F-1mgDST with the best sensitivity and specificity for detecting T2D patients with VFx.

P values <.05 were considered significant.

Results

Characteristics of T2D subjects and controls are reported in Table 1. Compared with controls, T2D patients showed reduced OC and β-CTX levels and an increased prevalence of hypertension, dyslipidemia, previous clinical fragility fractures, and asymptomatic VFx, despite increased spinal and femoral BMD. No differences were found between patients and controls in calcium excretion and creatinine clearance levels, propensity to falls, daily calcium intake, and prevalence of the different GR polymorphisms. On the contrary, T2D patients showed higher F-1mgDST and lower UFE levels than controls, whereas ACTH and UFF were comparable. The prevalence of retinopathy, nephropathy, and neuropathy was 5.1, 5.1, and 3.3%, respectively.

Comparisons between T2D patients with and without VFx and between controls with and without VFx are reported in Table 2. The prevalence of hypertension and dyslipidemia was similar in fractured and non-fractured T2D subjects. The use of loop diuretics, thiazide diuretics, and statins was comparable between fractured (2.9, 20.6, and 17.6%, respectively) and non-fractured T2D patients (1.5%, $P = .64$; 15.4%, $P = .51$; 30.8%, $P = .16$, respectively). The number of patients using insulin, dipeptidyl peptidase 4 inhibitors, glucagon-like peptide-1 agonists, metformin, and sulfonylureas was comparable between fractured (20.6, 23.5, 11.8, 64.7, and 5.9%, respectively) and non-fractured (18.5%, $P = .79$; 23.1%, $P = .96$; 7.7%, $P = .49$; 66.2%, $P = .89$; 13.8%, $P = .33$, respectively) patients. No subjects had hypoglycemic episodes during the 6 months before enrollment. The number of patients with a history of symptomatic (n = 7), and/or severe (n = 2), and/or frequent (n = 4), and/or unawareness of (n = 4) hypoglycemia during the 24 months before study enrollment was similar in fractured (5.9, 2.9, 5.9, and 5.9%, respectively) and non-fractured (7.7, 1.5, 3.1, and 3.1%, respectively) patients.

Compared to T2D subjects without VFx, in fractured T2D patients the BMD at FN was reduced, and the prevalence of retinopathy (15.4 vs 1.8%; $P = .04$) and of the heterozygous variant of N363S polymorphism was in-

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Table 1. General Characteristics of T2D and Non-T2D Subjects

Parameters	T2D Subjects	Non-T2D Subjects	*P*
n	99	107	
Age, y	65.7 ± 7.3 (52–80)	64.5 ± 8.2 (50–80)	.20
BMI, kg/m^2	29.5 ± 4.8 (21–39.9)	28.6 ± 4.7 (20.1–39.8)	.22
Years from menopause	14.0 ± 7.4 (2–30)	12.6 ± 8.1 (2–30)	.21
Smokers	16 (16.2)	28 (26.2)	.08
Patients experiencing 1/2 falls[a]	10/2 (10.1/2.0)	5/2 (4.7/1.9)	.46
Daily calcium intake, mg	675.4 ± 207 (330–1270)	635 ± 159 (364–950)	.22
Patients with hypertension	64 (64.6)	44 (41.5)	.001
Patients with dyslipidemia	47 (47.5)	30 (28.0)	.004
T2D duration, y	10.1 ± 7 (1–30)		
HbA1c, %	6.8 ± 0.7 (5.3–8)	5.4 ± 0.5 (4–6.0)	<.0001
Calcium, mg/dL	9.3 ± 0.4 (8.5–10)	9.3 ± 0.3 (8.6–10)	.17
25-OH vitamin D, ng/mL	19.2 ± 10.8 (12–61.4)	18.4 ± 7.5 (12–35)	.62
ALP, U/L	70.6 ± 16.5 (37–105)	71.2 ± 15.6 (45–105)	.82
24-h urinary calcium excretion, mg	133.6 ± 77.7 (71.5–385)	143.0 ± 80.3 (70–380.9)	.418
Creatinine clearance, mL/min	90.7 ± 26.7 (61–144.8)	90.2 ± 19.4 (60.5–145)	.861
ACTH, pg/mL	16.9 ± 11 (5–54.2)	16.4 ± 9.2 (5–51.4)	.72
F-1mgDST, μg/dL	1.21 ± 0.44 (0.4–1.8)	1.06 ± 0.42 (0.4–1.8)	.01
UFF, μg/24 h	19.2 ± 10.4 (4.7–54.7)	20.4 ± 9.5 (4.2–43)	.37
UFE, μg/24 h	87 ± 22.3 (33.7–166.3)	94.8 ± 18.2 (35–121)	.02
R-UFF/UFE	0.22 ± 0.11 (0.06–0.59)	0.20 ± 0.10 (0.08–0.45)	.22
OC, ng/mL[b]	4.9 ± 3.2 (−0.8 to 13.8)	10.6 ± 6.4 (−0.8 to 22.1)	<.0001
β-CTX, ng/mL[b]	0.14 ± 0.08 (−0.03 to 0.37)	0.28 ± 0.12 (−0.12 to 0.55)	<.0001
BMD LS, Z-score	0.78 ± 1.43 (−3.6 to 4.0)	0.16 ± 1.28 (−2.2 to 4.3)	.001
BMD LS, T-score	−0.80 ± 1.43 (−5.3 to 2.9)	−1.39 ± 1.27 (−4.1 to 2.8)	.002
BMD FN, Z-score	0.32 ± 0.98 (−1.8 to 2.6)	−0.03 ± 0.87 (−1.8 to 2.7)	.008
BMD FN, T-score	−1.06 ± 1.08 (−3.4 to 1.4)	−1.45 ± 0.91 (−3.1 to 1.4)	.006
Patients with previous clinical fragility Fx	17 (17.2)	7 (6.5)	.02
Patients with VFx	34 (34.3)	20 (18.7)	.01
*Bcl*I			.29
WT	61 (61.6)	55 (51.4)	
Heterozygous variant	33 (33.3)	47 (43.9)	
Homozygous variant	5 (5.1)	5 (4.7)	
N363S			.67
WT	8 (8.1)	7 (6.6)	
Heterozygous variant	0 (0.0)	0 (0.0)	
Homozygous variant	91 (91.9)	100 (93.5)	

Abbreviations: ALP, alkaline phosphatase; Fx, fracture; WT, wild type. Data are expressed as mean ± SD (range) or absolute number (percentage). Z-score represents the number of standard deviations above or below the mean for the patient's age, sex and ethnicity. T-score represents the number of standard deviations above or below the mean for a healthy 30-year-old adult of the same sex and ethnicity as the patient. Normal values: ALP, 35–105 U/L; ACTH, 5–55 pg/mL; F-1mgDST, <1.8 μg/dL; UFF, 3–43 μg/24-h; UFE, 15–122 μg/24 h; OC, 5–25 ng/mL; β-CTX, 0.14–1.35 ng/mL. SI conversion factors: serum cortisol × 27.59; ACTH × 0.22; urinary cortisol × 2.77; calcium × 0.25; 25-OH vitamin D × 2.5; OC × 1; β-CTX × 1.

[a] Data are referred to the last year before the study inclusion.

[b] OC and β-CTX levels were measured in 48 subjects with T2D and in 48 without T2D.

creased. No difference was found between fractured and non-fractured T2D patients in terms of BMD at LS, OC and β-CTX mean levels, prevalence of homozygous and/or heterozygous *Bcl*I GR variants, nephropathy (11.5 vs 3.6%; $P = .32$) and neuropathy (3.8 vs 3.8%; $P = 1$), 24-hour urinary calcium excretion, creatinine clearance, daily calcium intake, propensity to fall, R-UFF/UFE, and mean levels of the parameters of cortisol secretion. However, as shown in Figure 1, when T2D patients were divided into tertiles of F-1mgDST levels (I tertile: F-1mgDST, 0.4–0.85 μg/dL, 11–23.5 nmol/L; II tertile: F-1mgDST, 0.9–1.45 μg/dL, 24.8–40 nmol/L; III tertile: F-1mgDST, 1.5–1.8 μg/dL, 41.4–50 nmol/L), the prevalence of VFx significantly increased from the lowest to the highest tertile (21.2, 33.3, and 48.5%, respectively; p for trend = 0.038), whereas no association was found between the tertiles of HbA1c and VFx prevalence (I, II, III tertiles: 47.1, 23.5, and 29.4%, respectively; p for trend = 0.122). The ROC curve analysis showed that the cutoffs of F-1mgDST with the best diagnostic sensitivity and specificity for detecting T2D patients with VFx were > 0.9 μg/dL (24.8 nmol/L) and ≥ 1.5 μg/dL (41.3 nmol/L), respectively. Indeed, 48 of the 65 non- fractured T2D patients had F-1mgDST ≤ 0.9 μg/dL (24.8 nmol/L; specificity 73.8%; $P = .04$), whereas 27 of the 34 fractured T2D patients had F-1mgDST ≥ 1.5 μg/dL (41.3 nmol/L; sensitivity, 79.4%; $P = .04$).

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Table 2. Characteristics of T2D and Non-T2D Subjects With and Without VFx

Parameters	T2D Subjects With VFx	T2D Subjects Without VFx	*P*	Non-T2D Subjects With VFx	Non-T2D Subjects Without VFx	*P*
n	34	65		20	87	
Age, y	66.4 ± 7.8 (52–78)	65.4 ± 7.2 (52–80)	.52	65.1 ± 9.7 (50–80)	64.3 ± 7.9 (50–80)	.72
BMI, kg/m^2	28.9 ± 4.0 (21–35.3)	29.8 ± 5.2 (21.3–39.9)	.40	28.2 ± 5.5 (21.6–37.6)	28.7 ± 4.6 (20–39.8)	.67
Years from menopause	14.9 ± 7.3 (2–29)	13.5 ± 7.4 (2–30)	.36	14.5 ± 9.0 (2–30)	12.1 ± 7.9 (2–30)	.23
Smokers	4[b] (11.8)	12[d] (18.5)	.57	9 (45.0)	19 (21.8)	.04
Patients experiencing 1/2 falls[a]	4/0 (11.7/0.0)	6/2 (9.2/3.1)	.523	3/1 (15.0/5.0)	2/1 (10.0/5.0)	.2
Daily calcium intake, mg	622 ± 175 (357–1043)	698 ± 217 (329–1271)	.17	671 ± 173 (407–907)	621.7 ± 153 (364–950)	.3
Patients with hypertension	24[b] (70.6)	40[d] (61.5)	.37	7 (35.0)	37 (43.0)	.62
Patients with dyslipidemia	13 (38.2)	34 (52.3)	.18	7 (35.0)	23 (26.4)	.43
Patients with previous fragility Fx	7 (20.6)	10[d] (15.4)	.51	3 (15.0)	4 (4.6)	.12
T2D duration, y	11.7 ± 6.5 (2–25)	9.2 ± 5.8 (2–30)	.13			
HbA1c, %	6.6 ± 0.7 (5.3–8.0)	6.9 ± 0.7 (5.3–8.0)	.10	5.4 ± 0.6 (4.0–6.0)	5.4 ± 0.5 (4.0–6.0)	.75
Calcium, mg/dL	9.3 ± 0.4 (8.5–10.0)	9.3 ± 0.4 (8.5–10.0)	.82	9.3 ± 0.4 (8.6–10)	9.2 ± 0.3 (8.6–10.0)	.50
25-OH vitamin D, ng/mL	20.1 ± 12.3 (12–55.5)	18.8 ± 10.1 (12–61.4)	.63	18.6 ± 6.0 (12.0–29.9)	18.4 ± 8.0 (12.0–35)	.91
ALP, U/L	72.1 ± 17.9 (46–105)	70.0 ± 15.9 (37–105)	.58	70.6 ± 18.1 (47–105)	71.3 ± 15.1 (45–105)	.86
24-h urinary calcium excretion, mg	135.9 ± 86.6 (71.5–385)	132.4 ± 73.4 (71.7–365)	.84	130.9 ± 74.6 (70–348)	146.5 ± 82.1 (72–380)	.44
Creatinine clearance, mL/min	88.4 ± 24.3 (61.7–144.8)	91.9 ± 28.0 (61–144.9)	.48	88.6 ± 20.0 (6 5–134)	90.5 ± 19.3 (60.5–145)	.74
ACTH, pg/mL	18.2 ± 12.3 (5–54.2)	16.3 ± 10.4 (5–54)	.40	13.0 ± 7.1 (5–32)	17.2 ± 9.5 (5–51.4)	.07
F-1mgDST, μg/dL	1.26 ± 0.47 (0.4–1.8)	1.19 ± 0.43 (0.4–1.8)	.42	1.08 ± 0.41 (0.4–1.8)	1.06 ± 0.4 (0.4–1.8)	.79
UFF, μg/24 h	16.9 ± 9.6 (5.0–53.1)	20.4 ± 10.7 (4.7–54.7)	.11	23.7 ± 11.7 (5–43)	19.7 ± 8.8 (4.2–43)	.09
UFE, μg/24 h	85.7 ± 18.8 (42.2–111.1)	87.7 ± 24.0 (33.7–166.3)	.72	96.0 ± 19.1 (53.5–120.4)	94.4 ± 18.1 (35–121.0)	.76
R-UFF/UFE	0.21 ± 0.10 (0.10–0.48)	0.23 ± 0.11 (0.06–0.59)	.61	0.23 ± 0.10 (0.09–0.45)	0.20 ± 0.08 (0.08–0.40)	.12
OC, ng/mL[b]	4.6 ± 3.4[c] (0.8–13.8)	5.2 ± 3.1[d] (0.8–10.4)	.62	9.9 ± 7.0 (1.7–21.5)	11.0 ± 6.3 (0.8–22.1)	.63
β-CTX, ng/mL[b]	0.11 ± 0.06[c] (0.05–0.26)	0.16 ± 0.09 (0.03–0.37)	.11	0.28 ± 0.11 (0.12–0.55)	0.28 ± 0.13 (0.12–0.48)	.78
BMD LS, Z-score	0.47 ± 1.58 (−3.6 to 4.0)	0.95 ± 1.34[d] (−1.4 to 3.9)	.12	0.17 ± 1.25 (−2.2 to 2.7)	0.16 ± 1.30 (−2.1 to 4.3)	.99
BMD LS, T-score	−1.20 ± 1.43 (−5.3 to 1.7)	−0.59 ± 1.40[d] (−3.2 to 2.9)	.05	−1.29 ± 1.44 (−3.6 to 2.2)	−1.41 ± 1.25 (−4.1 to 2.8)	.69
BMD FN, Z-score	0.02 ± 1.03 (−1.8 to 2.6)	0.47 ± 0.92[d] (−1.2 to 2.1)	.03	−0.39 ± 0.86 (−1.8 to 1.9)	0.05 ± 0.86 (−1.3 to 2.7)	.04
BMD FN, T-score	−1.44 ± 1.04 (−3.4 to 0.7)	−0.87 ± 1.05[d] (−2.9 to 1.4)	.01	−1.89 ± 0.96 (−3.1 to 0.4)	−1.34 ± 0.87 (−2.9 to 1.4)	.015
*Bcl*I			.96			.82
WT	21 (61.8)	40 (61.5)		9 (45.0)	46 (52.9)	
Heterozygous variant	11 (32.4)	22 (33.8)		10 (50.0)	37 (42.5)	
Homozygous variant	2 (5.9)	3 (4.6)		1 (5.0)	4 (4.6)	
N363S			.02			.02
WT	28 (82.4)	63 (96.9)		16 (80)	84 (96.6)	
Heterozygous variant	6 (17.6)	2 (3.1)		4 (20)	3 (3.4)	
Homozygous variant	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	

Abbreviations: ALP, alkaline phosphatase; Fx, fracture; WT, wild type. Data are expressed as mean ± SD (range) or absolute number (percentage). Normal values: ALP, 35–105 U/L; OC, 5–25 ng/mL; β-CTX, 0.14–1.35 ng/mL. Z-score represents the number of standard deviations above or below the mean for the patient's age, sex and ethnicity. T-score represents the number of standard deviations above or below the mean for a healthy 30-year-old adult of the same sex and ethnicity as the patient. SI conversion factors: serum cortisol × 27.59; ACTH × 0.22; urinary cortisol × 2.77; calcium × 0.25; 25-OH vitamin D × 2.5; OC × 1; β-CTX × 1.

[a] Data are referred to the last year before the study inclusion.

[b] OC and β-CTX levels were measured in 48 subjects with T2D and in 48 without T2D.

[c] $P < .05$ vs controls with VFx.

[d] $P < .05$ vs controls without VFx.

Similar to T2D patients, in controls with VFx, the BMD at FN was reduced, and the prevalence of the heterozygous variant of N363S polymorphism increased. Fractured and non- fractured controls were comparable in terms of OC, β-CTX, BMD at LS and R-UFF/UFE levels, mean levels of the parameters of cortisol secretion, prevalence of homozygous and/or heterozygous *Bcl*I GR variants, 24-hour urinary calcium excretion, creatinine clearance, daily calcium intake, and propensity to fall. At variance with T2D patients, in fractured control subjects, prevalence of the smoking habit was increased as compared with their non-fractured counterparts. In controls, no association was found between the tertiles of F-1mgDST and VFx prevalence (I, II, and III tertiles: 19.4, 16.7, and 20%, respectively; p for trend = 0.93), and the ROC curve analysis did not show statistically significant cutoffs of F-1mgDST for detecting VFx.

Considering the whole population, cortisol secretion and R-UFF/UFE levels were associated with neither BMD nor HbA1c levels. However, OC levels were inversely associated with F-1mgDST levels ($R = -0.25$; $P = .03$). This association was confirmed in control subjects ($R = -0.338$; $P = .04$) but not in T2D patients (Figure 2).

In the whole sample of T2D patients and controls, HbA1c levels were inversely associated with β-CTX ($R = -0.51$; $P < .0001$) and OC levels ($R = -0.37$; $P = .001$; Figure 3). These associations were absent when controls and T2D patients were separately considered.

The logistic regression analysis showed that in T2D patients, the presence of VFx was significantly associated with the sensitizing variant of N363S polymorphism and with tertiles of F-1mgDST levels, even after adjusting for the history of fragility fractures, BMI, age, and spinal BMD (Table 3). Because the pathophysiological mechanisms of osteoporosis may be different in postmenopausal and senile osteoporosis (36), we assessed the same associations even after adjusting for age ≤ 65 or > 65 years,

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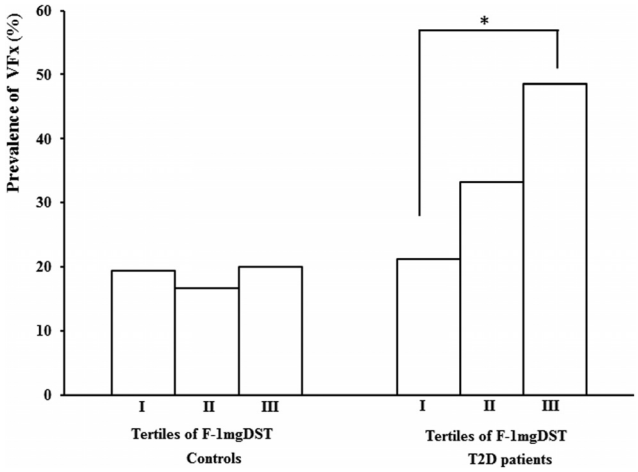

Figure 1. Association between F-1mgDST levels and the prevalence of VFx in patients with T2D and in control subjects. Diabetic patients and control subjects are divided into tertiles on the basis of the increasing F-1mgDST. F-1mgDST of T2D patients: I tertile, 0.4–0.85 μg/dL; II tertile, 0.9–1.45 μg/dL; III tertile, 1.5–1.8 μg/dL. F-1mgDST of control subjects: I tertile, 0.4–0.9 μg/dL; II tertile, 0.91–1.49 μg/dL; III tertile, 1.5–1.8 μg/dL. SI conversion factor: serum cortisol × 27.59. *, In T2D but not in control subjects, the prevalence of VFx increases significantly from I tertile to III tertile (p for trend = 0.038).

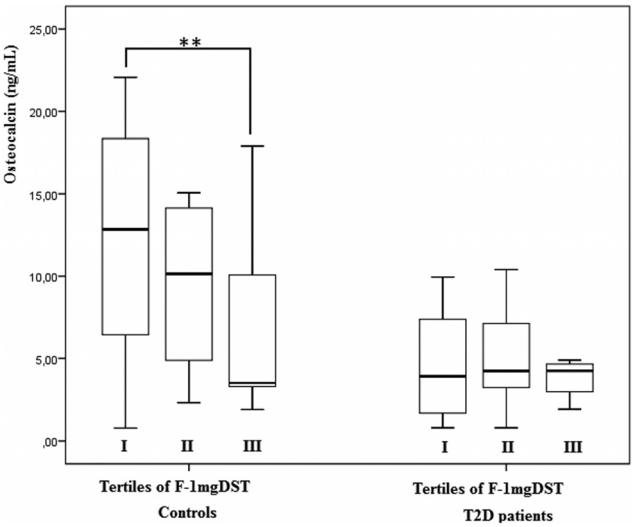

Figure 2. Association between F-1mgDST levels and OC levels in patients with T2D and in control subjects. Diabetic patients and control subjects are divided into tertiles on the basis of the increasing cortisol levels after F-1mgDST. F-1mgDST of T2D patients: I tertile, 0.4–0.85 μg/dL; II tertile, 0.9–1.45 μg/dL; III tertile, 1.5–1.8 μg/dL. F-1mgDST of control subjects: I tertile, 0.4–0.9 μg/dL; II tertile, 0.91–1.49 μg/dL; III tertile, 1.5–1.8 μg/dL. SI conversion factor: serum cortisol × 27.59. **, In control subjects, F-1mgDST was associated with OC (R = −0.338; P = .04), whereas in T2D patients this association was absent. Controls: OC 25th and 75th percentiles, mean ± SEM—I tertile, 6.2, 18.7, 12.8 ± 1.7 ng/mL, respectively; II tertile, 4.5, 14.2, 9.0 ± 1.3 ng/mL, respectively; III tertile, 2.6, 14.0, 7.3 ± 3.0 ng/mL, respectively. T2D patients: OC 25th and 75th percentiles, mean ± SEM—I tertile, 1.6, 7.6, 4.7 ± 0.9 ng/mL, respectively; II tertile, 2.9, 7.4, 5.2 ± 0.8 ng/mL, respectively; III tertile, 2.5, 4.9, 4.5 ± 1.0 ng/mL, respectively.

and we obtained the same results (data not shown). In controls, the same analysis showed that the VFx presence was associated with the N363S polymorphism but not with F-1mgDST levels (data not shown).

Discussion

This study was aimed to assess the role of cortisol secretion and sensitivity in T2D-related osteoporosis. Our data confirm that in T2D the prevalence of fragility fractures is increased despite an increased BMD, cortisol secretion is enhanced, and bone turnover is reduced. Moreover, VFx were associated with both the heterozygous sensitizing variant of N363S polymorphism and the degree of cortisol secretion.

The finding of an increased fracture risk in T2D despite an increased BMD confirms previous reports (1–8). However, most studies reported that the risk of fractures was high in T2D patients with chronic complications and/or scarce metabolic control (2, 8). The present finding that a VFx is present in the 30% of T2D outpatients with good metabolic control and without diabetic complications is of clinical importance because it suggests the need of paying attention to bone involvement regardless of the severity of the diabetic disease.

Among the factors associated with VFx, in our fractured T2D patients, FN BMD was reduced and the prevalence of retinopathy was increased more than in non-fractured patients. This is in keeping with previous data showing that T2D patients with fractures have a reduced femoral BMD (37) and an increased prevalence of diabetic retinopathy (15, 16), compared with non-fractured patients. At variance with previous studies (3, 5), the glycometabolic control was comparable between fractured and non-fractured T2D patients. This discordance may depend on the fact that only patients with HbA1c ≤ 8.0% were included in the present study. This may also justify the relatively higher OC levels that we found in T2D subjects in respect to previous data (10) and may explain why some T2D patients showed OC levels within the range of

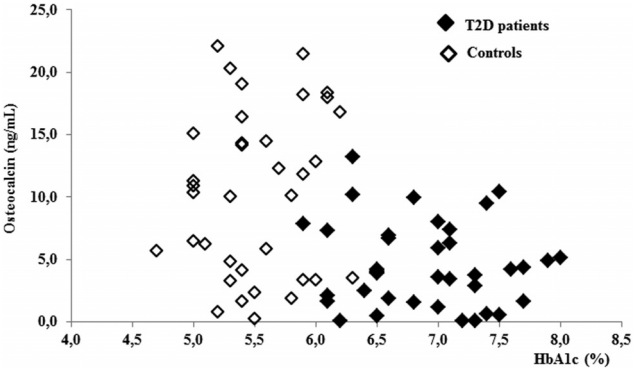

Figure 3. Association between OC and HbA1c levels in the whole population of T2D patients and control subjects. In the whole sample of T2D patients and controls, HbA1c levels were inversely associated with OC levels (R = −0.37; P = .001).

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Table 3. Factors Independently Associated With VFx in T2D by Logistic Regression Analysis

Factor	OR	95% CI	*P*
Age (1-y increase)	1.0	0.9–1.1	.8
BMI (1 kg/m^2 decrease)	1.0	0.9–1.1	.95
BMD LS (1 SD Z-score decrease)	1.4	0.9–2.0	.06
Previous fragility Fx (presence vs absence)	1.0	0.3–3.6	.97
N363S heterozygous variant (presence vs absence)	12.5	1.8–88.7	.01
Tertiles of F-1mgDST levels (I vs II vs III tertile)[a]	2.1	1.1–4.1	.031

Abbreviations: Fx, fracture; OR, odds ratio; CI, confidence interval.

[a] The T2D diabetic patients were divided into tertiles on the basis of F-1mgDST levels: I tertile, F-1mgDST 0.4–0.85 μg/dL; II tertile: 0.9–1.45 μg/dL; III tertile: 1.5–1.8 μg/dL.

controls (Figure 3). The finding that bone apposition and resorption (as mirrored by OC and β-CTX levels, respectively) were inversely associated with HbA1c levels is in keeping with the idea that chronic hyperglycemia may have a pathophysiological role in diabetes-related osteoporosis. However, these findings must be viewed cautiously, considering the heterogeneity of the OC levels in controls and the large overlap of OC levels between T2D patients and controls. It is not possible to exclude, therefore, that other factors might have influenced these results.

From a pathophysiological point of view, the present findings may be interesting. Indeed, in keeping with a previous study on complicated T2D inpatients (28), our data also show that in T2D outpatients adequately compensated and without diabetic complications, cortisol secretion is slightly enhanced and that the degree of cortisol secretion is directly associated with the prevalence of VFx. Apparently in contradiction, we did not find differences between T2D patients with and without VFx in mean levels of the parameters of cortisol secretion. However, it must be considered that, due to the inclusion criteria, all T2D patients included had normal cortisol secretion. Therefore, in T2D the relation between skeletal health and cortisol levels might be related to the degree of cortisol secretion, even in the absence of hypercortisolism. In T2D patients, but not in controls, we found two cutoffs of F-1mgDST (≤0.9 μg/dL, 24.8 nmol/L; and ≥1.5 μg/dL, 41.3 nmol/L), both within the normal range, that may predict the absence and the presence of VFx with a 79.8% sensitivity and a 73.8% specificity, respectively.

The lack of an inverse association between OC and cortisol levels in T2D subjects would also conflict with the hypothesis of a direct effect of cortisol secretion on bone apposition. Nevertheless, the low OC levels in most of our T2D subjects could have masked the relationship between cortisol secretion and bone apposition, which, at variance, was found in control subjects.

A second main finding of the present study is that VFx are independently associated with increased cortisol sensitivity due to the N363S-sensitizing polymorphism of the GR in both T2D patients and controls. Although the N363S polymorphisms were found to potentially influence bone metabolism in osteoporosis (25–27) and disease control in diabetes (30–31), no data were available so far regarding their potential role in T2D-related osteoporosis. The direct association between the sensitizing N363S polymorphism of GR with VFx is in accordance with the idea that a cortisol secretion at the upper limit of normal range may play a role in the pathogenesis of the T2D-related osteoporosis.

At variance with data regarding cortisol secretion and sensitivity, cortisol availability, measured by the R-UFF/UFE, did not seem to influence bone metabolism in T2D because the R-UFF/UFE, the parameter commonly used to evaluate 11HSD type 2 activity (24), was not associated with the presence of VFx in both T2D patients and controls. However, this enzyme is expressed mainly at the renal level, whereas in bone the conversion from cortisone to cortisol is mediated by 11HSD type 1 (11HSD1), whose activity is not well mirrored by R-UFF/UFE (22). Interestingly, drugs inhibiting 11HSD1 are under evaluation for their potential role in the treatment of T2D (38). If osteoporosis in T2D were related, at least in part, to the degree of cortisol secretion and/or sensitivity, the treatment with an 11HSD1 inhibitor could improve diabetic control and reduce fracture risk at the same time.

Overall, these findings must be considered cautiously due to several limits of the study itself. First, the cross-sectional design allows investigation for association and not for causality, and most importantly, the study is not randomized and results for the controls relative to the T2D patients are rather heterogeneous. However, in this study the diabetic and nondiabetic outpatients were consecutively enrolled. This design allowed us to have a "real life" picture of the prevalence of VFx in T2D outpatients and to investigate the potential role of the assessment of cortisol sensitivity and secretion in the evaluation of T2D patients at risk for osteoporosis. In this regard, the finding of two possible cutoffs for F-1mgDST for detecting T2D patients without VFx and with VFX might be useful in clinical practice.

Second, in this case-control study, T2D patients and controls may have been different not only with respect to hyperlipidemia and hypertension, but probably also for other unknown factors. For example, the possible influence of the comorbidities and of the drugs used for treating diabetes, hypertension, and dyslipidemia cannot be ex-

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cluded (39, 40), even if the prevalence of hypertension and dyslipidemia and the different agents used was comparable between fractured and non-fractured T2D patients. In addition, we excluded patients with scarcely compensated diabetes, and therefore the role of glycemic control may have been underscored. On the other hand, this exclusion criterion allowed us to minimize the effect on skeletal tissue of a definitely increased cortisol secretion (3, 8).

Third, we did not repeat the determination of F-1mgDST, and therefore we could not directly test the reproducibility of F-1mgDST in our sample. However, the main reasons for a reduced reproducibility of F-1mgDST are the administration of drugs or the presence of diseases that could influence dexamethasone metabolism, two conditions that were among the exclusion criteria of the study.

In conclusion, this study suggests that in postmenopausal female outpatients with T2D: 1) the prevalence of VFx is increased despite an increased BMD, and bone turnover is reduced; and 2) the presence of VFx is associated with the degree of cortisol secretion and with the sensitizing variant of the N363S polymorphism of the GR.

Further studies could be designed to ascertain whether the parameters of cortisol secretion and sensitivity may be used for personalizing the therapy in T2D-related osteoporosis.

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References

1. **Nicodemus KK, Folsom AR, Iowa Women's Health Study.** Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women. *Diabetes Care*. 2001;24:1192–1197.
2. **Hofbauer LC, Brueck CC, Singh SK, Dobnig H.** Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res*. 2007;22:1317–1328.
3. **Vestergaard P.** Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis. *Osteoporos Int*. 2007;18:427–444.
4. **Janghorbani M, Van Dam RM, Willett WC, Hu FB.** Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol*. 2007;166:495–505.
5. **Yamamoto M, Yamaguchi T.** Diabetic patients have an increased risk of vertebral fractures independent of BMD or diabetic complications. *J Bone Miner Res*. 2009;24:702–709.
6. **Schwartz AV, Vittinghoff E, Bauer DC, et al.** Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA*. 2011;305:2184–2192.
7. **Hothersall EJ, Livingstone SJ, Looker HC, et al.** Contemporary risk of hip fracture in type 1 and type 2 diabetes: a national registry study from Scotland. *J Bone Miner Res*. 2014;29:1054–1060.
8. **Burghardt AJ, Issever AS, Schwartz AV, et al.** High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2010;95:5045–5055.
9. **Carnevale V, Morano S, Fontana A, et al.** Assessment of fracture risk by the FRAX algorithm in men and women with and without type 2 diabetes mellitus: a cross-sectional study. *Diabetes Metab Res Rev*. 2014;30:313–322.
10. **Gennari L, Merlotti D, Valenti R, et al.** Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. *J Clin Endocrinol Metab*. 2012;97:1737–1744.
11. **Gaudio A, Privitera F, Battaglia K, et al.** Sclerostin levels associated with inhibition of the Wnt/β-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2012;97:3744–3750.
12. **Hamann C, Kirschner S, Günther KP, Hofbauer LC.** Bone, sweet bone–osteoporotic fractures in diabetes mellitus. *Nat Rev Endocrinol*. 2012;8:297–305.
13. **Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T.** Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab*. 2008;93:1013–1019.
14. **Schwartz AV, Garnero P, Hillier TA, et al.** Pentosidine and increased fracture risk in older adults with type 2 diabetes. *J Clin Endocrinol Metab*. 2009;94:2380–2386.
15. **Kim JH, Jung MH, Lee JM, Son HS, Cha BY, Chang SA.** Diabetic peripheral neuropathy is highly associated with nontraumatic fractures in Korean patients with type 2 diabetes mellitus. *Clin Endocrinol (Oxf)*. 2012;77:51–55.
16. **Muñoz-Torres M, Reyes-García R, García-Martin A, et al.** Ischemic heart disease is associated with vertebral fractures in patients with type 2 diabetes mellitus. *J Diabetes Investig*. 2013;4:310–315.
17. **Chiodini I, Scillitani A.** Role of cortisol hypersecretion in the pathogenesis of osteoporosis [in Italian]. *Recenti Prog Med*. 2008;99:309–313.
18. **Chiodini I, Mascia ML, Muscarella S, et al.** Subclinical hypercortisolism among outpatients referred for osteoporosis. *Ann Intern Med*. 2007;147:541–548.
19. **Reynolds RM, Dennison EM, Walker BR, et al.** Cortisol secretion and rate of bone loss in a population-based cohort of elderly men and women. *Calcif Tissue Int*. 2005;77:134–138.
20. **Liu YZ, Dvornyk V, Lu Y, et al.** A novel pathophysiological mechanism for osteoporosis suggested by an in vivo gene expression study of circulating monocytes. *J Biol Chem*. 2005;280:29011–29016.
21. **Cooper MS, Blumsohn A, Goddard PE, et al.** 11β-hydroxysteroid dehydrogenase type 1 activity predicts the effects of glucocorticoids on bone. *J Clin Endocrinol Metab*. 2003;88:3874–3877.
22. **Tomlinson JW, Walker EA, Bujalska IJ, et al.** 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev*. 2004;25:831–866.
23. **Hwang JY, Lee SH, Kim GS, et al.** HSD11B1 polymorphisms predicted bone mineral density and fracture risk in postmenopausal women without a clinically apparent hypercortisolemia. *Bone*. 2009;45:1098–1103.
24. **van Rossum EF, Voorhoeve PG, te Velde SJ, et al.** The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. *J Clin Endocrinol Metab*. 2004;89:4004–4009.
25. **Szappanos A, Patócs A, Tõke J, et al.** *Bcl*I polymorphism of the glucocorticoid receptor gene is associated with decreased bone min-

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eral density in patients with endogenous hypercortisolism. *Clin Endocrinol (Oxf)*. 2009;71:636–643.
26. **Morelli V, Donadio F, Eller-Vainicher C, et al.** Role of glucocorticoid receptor polymorphism in adrenal incidentalomas. *Eur J Clin Invest*. 2010;40:803–811.
27. **van Schoor NM.** Serum fasting cortisol in relation to bone, and the role of genetic variations in the glucocorticoid receptor. *Clin Endocrinol (Oxf)*. 2007;67:871–878.
28. **Chiodini I, Adda G, Scillitani A, et al.** Cortisol secretion in patients with type 2 diabetes: relationship with chronic complications. *Diabetes Care*. 2007;30:83–88.
29. **Chiodini I, Torlontano M, Scillitani A, et al.** Association of subclinical hypercortisolism with type 2 diabetes mellitus: a case-control study in hospitalized patients. *Eur J Endocrinol*. 2005;153:837–844.
30. **van Rossum EF, Koper JW, Huizenga NA, et al.** A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. *Diabetes*. 2002;51:3128–3134.
31. **Manenschijn L, van den Akker EL, Lamberts SW, van Rossum EF.** Clinical features associated with glucocorticoid receptor polymorphisms. An overview. *Ann NY Acad Sci*. 2009;1179:179–198.
32. **Cairoli E, Eller-Vainicher C, Ulivieri FM, et al.** Factors associated with bisphosphonate treatment failure in postmenopausal women with primary osteoporosis. *Osteoporos Int*. 2014;25:1401–1410.
33. **Eller-Vainicher C, Chiodini I, Santi I, et al.** Recognition of morphometric vertebral fractures by artificial neural networks: analysis from GISMO Lombardia Database. *PLoS One*. 2011;6:e27277.
34. **Fustinoni S, Polledri E, Mercadante R.** High-throughput determination of cortisol, cortisone, and melatonin in oral fluid by on-line turbulent flow liquid chromatography interfaced with liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2013;27:1450–1460.
35. **Genant HK, Wu CY, van Kuijk C, Nevitt MC.** Vertebral fracture assessment using a semiquantitative technique. *J Bone Miner Res*. 1993;8:1137–1148.
36. **Riggs BL, Melton LJ 3rd.** Evidence for two distinct syndromes of involutional osteoporosis. *Am J Med*. 1983;75:899–901.
37. **Napoli N, Strotmeyer ES, Ensrud KE, et al.** Fracture risk in diabetic elderly men: the MrOS study. *Diabetologia*. 2014;57:2057–2065.
38. **Rosenstock J, Banarer S, Fonseca VA, et al.** The 11-β-hydroxysteroid dehydrogenase type 1 inhibitor INCB13739 improves hyperglycemia in patients with type 2 diabetes inadequately controlled by metformin monotherapy. *Diabetes Care*. 2010;33:1516–1522.
39. **Ilic' K, Obradovic' N, Vujasinovic'-Stupar N.** The relationship among hypertension, antihypertensive medications, and osteoporosis: a narrative review. *Calcif Tissue Int*. 2013;92:217–227.
40. **McCandless LC.** Statin use and fracture risk: can we quantify the healthy-user effect? *Epidemiology*. 2013;24:743–752.

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