Rosiglitazone Decreases Bone Mineral Density and Increases Bone Turnover in Postmenopausal Women With Type 2 Diabetes Mellitus

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Context: Postmenopausal status and type 2 diabetes mellitus (T2DM) are independent risk factors for fractures. An increased fracture risk has been observed with rosiglitazone (RSG), a thiazolidinedione, in patients with T2DM.

Design and Setting: This was a randomized, double-blind study in postmenopausal women with T2DM. A 52-week double-blind phase (RSG or metformin [MET]) was followed by a 24-week open-label phase, during which time all patients received MET.

Main Outcome Measures: The primary endpoint was to assess the mean percentage change in bone mineral density (BMD) at the femoral neck (FN) by dual-energy x-ray absorptiometry from baseline to week 52 in the RSG treatment group. Key secondary objectives included assessment of changes in BMD at the total hip, trochanter, and lumbar spine and to evaluate RSG effects on bone turnover markers.

Results: From baseline to week 52, RSG was associated with a reduction in FN BMD by dual-energy x-ray absorptiometry (−1.47%). During the open-label phase (weeks 52–76), no further loss in FN BMD was observed. A decrease in BMD occurred at the total hip during RSG or MET treatment at 52 weeks (−1.62 and −0.72%, respectively). Total hip BMD loss by RSG was attenuated after switching to MET and was similar between treatment groups at the end of the open-label phase. From baseline to week 52, bone turnover markers significantly increased with RSG compared with MET, but decreased significantly during the open-label phase.

Conclusions: RSG for 52 weeks in postmenopausal women with T2DM was associated with small reductions in FN, total hip, and lumbar spine BMD and increased bone turnover markers. These effects are attenuated after cessation of RSG treatment. **(*J Clin Endocrinol Metab* 98: 1519–1528, 2013)**

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Abbreviations: AE, adverse event; BMD, bone mineral density; BSAP, bone-specific alkaline phosphatase; CTX, C-terminal crosslinking telopeptide of type I collagen; DXA, dual-energy x-ray absorptiometry; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; HOMA-S, homeostasis model of assessment for insulin sensitivity; MET, metformin; 25-OHD, 25-hydroxyvitamin D; PINP, procollagen type I N-terminal propeptide; PPAR-γ, peroxisome proliferator-activated receptor-γ; QC, quality control; RSG, rosiglitazone; SAE, serious AE; T2DM, type 2 diabetes mellitus; TZD, thiazolidinedione.

Postmenopausal women are at a higher risk of osteoporosis and subsequent fractures than premenopausal women and men, making them a more vulnerable population to interactions with additional risk factors for fracture. Women with type 2 diabetes mellitus (T2DM) have normal or higher bone mineral density (BMD) for their age, but approximately double the overall risk of skeletal fractures compared with nondiabetic subjects (1–6). Epidemiological studies corroborate T2DM in women as an independent risk factor for fracture (7), and it was proposed that diabetes should be included as an independent variable in fracture assessment by the World Health Organization Fracture Risk Assessment Tool (FRAX) (8).

Given the association between T2DM and fracture risk, investigating the effects of widely used oral antidiabetic agents, such as metformin (MET) and thiazolidinediones (TZDs; for example, rosiglitazone [RSG] and pioglitazone), on bone mass and turnover is clinically relevant for patients and practitioners. In ADOPT (A Diabetes Outcomes Progression Trial), a post hoc analysis indicated that women treated with RSG experienced an increased risk of fractures in comparison with those receiving MET or glyburide, with most fractures reported in the hand, proximal humerus, and foot (9). In the RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycemia in Diabetes) study, this finding was confirmed with the analysis of self-reported adverse events (AEs) (10). Similarly, increased incidence of distal extremity fractures in women receiving long-term treatment with pioglitazone for T2DM was reported in a post hoc analysis of the PROactive (Prospective Pioglitazone Clinical Trial in Macrovascular Events) trial (11, 12).

TZDs act as ligands for peroxisome proliferator-activated receptor-γ (PPAR-γ) and directly regulate genes involved in glucose homeostasis and adipogenesis. PPAR-γ is expressed in bone marrow stromal cells, adipocytes, osteoblasts, and osteoclasts (13–16). Although the exact causative factors responsible for the effects of TZDs on bone are not certain, a number of mechanisms have been proposed, including an increase in adipocyte formation at the expense of osteoblast production (17), promotion of osteoclast differentiation and action (18–20), and stimulation of osteoblast cell apoptosis (21). Other potential actions of TZDs include effects on adipokines and inflammatory cytokines (22–24), activation of the *Wnt* signaling pathway (25, 26), changes in energy metabolism affecting the skeleton (27), prolonged hyperglycemia (28–34), inhibition of aromatase, and decreased estrogen synthesis (35). MET, a dimethyl-biguanide, stimulated osteoblasts in culture (36), but clinical data are more inconsistent, with studies showing that MET has varied effects on bone turnover markers (37) and little influence on fracture rates (38).

This study focuses primarily on the measurement of BMD at the femoral neck, a skeletal site that is easily measured and has substantial (up to 75%) cortical bone (39). Femoral neck BMD may be more relevant to the diabetic population than lumbar spine BMD in that fractures in patients treated with RSG are typically at cortical skeletal sites, such as the proximal humerus, hands, and feet (9). The aims of this study are to evaluate the effects of RSG on BMD in postmenopausal women with T2DM and to evaluate the potential reversibility of changes in bone mass and turnover on cessation of RSG treatment, thereby providing insight into the clinical significance of the effect of RSG on fracture risk.

Patients and Methods

Study design and patients

The methodology has been described in detail elsewhere (40). A brief outline of the key aspects is included here. This was a randomized, double-blind, multicenter study consisting of 3 phases: a screening phase, a 52-week double-blind treatment phase (patients randomized to RSG or MET), and a 24-week open-label follow-up phase in which RSG was discontinued and all subjects received MET. The main inclusion criteria were: women 55–80 years of age; >5 years postmenopausal; T2DM; BMD T-score greater than −2.5 at the total hip, femoral neck, and lumbar spine; and prior antidiabetic therapy with diet and exercise alone or monotherapy (non-TZD) for > 2 weeks within the past 12 weeks. Subjects were required to have a glycosylated hemoglobin A1c (HbA1c) ≤ 9.0% if drug-naive and ≤ 8.5% if on prior monotherapy. Exclusion criteria included: type 1 diabetes mellitus; history of diabetic ketoacidosis or uncontrolled hypertension; simultaneous treatment with 2 or more antidiabetic agents within the past 12 weeks; and previous treatment with estrogens and other bone-active drugs. Randomized patients were prescribed calcium (500–1000 mg elemental calcium daily) and vitamin D (at least 400 IU daily) supplements once daily throughout the study. Dual-energy x-ray absorptiometry (DXA) assessments of the lumbar spine and hip were performed at screening and at weeks 16, 28, 52, and 76. The scans were sent to a central facility for quality control (QC), analysis, and review.

Study medication taken during the double-blind treatment period was blinded to the subjects and the investigator. Blinding was maintained during this phase by use of the double-dummy technique. Blinded study medication was overencapsulated to appear the same. To obtain similar glycemic control between groups, a titration algorithm of study medications was used, and the study was controlled for concomitant antidiabetic medications. At baseline, subjects on prior monotherapy were switched to study medication. RSG was initiated at a total daily dose of 4 mg and force-titrated to 8 mg. MET was begun at 1000 mg and force-titrated to 2000 mg. From weeks 8 to 16, subjects with mean daily glucose >6.1 mmol/L at the maximum tolerated doses of blinded RSG or MET re-

ceived open-label sulfonylurea. After 4 months of double-blind study medication, subjects with HbA1c >7.5% at the maximum dose of medication could up-titrate or add open-label sulfonylurea at the discretion of the investigator. After 52 weeks, subjects were force-titrated to a total daily dose of 2000 mg MET in an open-label manner. Subjects with poor glycemic control at the maximum tolerated dose of MET were up-titrated to additional open-label sulfonylurea at the discretion of the investigator (40).

Randomization was computer-generated with central randomization by region. To achieve balance between treatment groups, eligible subjects were stratified by prior antidiabetic therapy and randomized in a 1:1 ratio to RSG or MET, within each stratum.

The primary objective was to assess the mean percentage change in BMD at the femoral neck by DXA from baseline to week 52 in the RSG treatment group. Secondary objectives included within and between treatment group comparisons of changes from baseline at prespecified time points in: femoral neck, lumbar spine, and total hip BMD as measured by DXA; serum bone-specific alkaline phosphatase (BSAP), serum procollagen type I N-terminal propeptide (PINP) and serum C-terminal crosslinking telopeptide of type I collagen (CTX); serum calcium, 25-hydroxyvitamin D (25-OHD), and PTH; and clinical safety. Exploratory objectives included within and between treatment group comparisons of change from baseline at prespecified time points in HbA1c, fasting plasma glucose (FPG), fasting plasma insulin, and insulin sensitivity measured by the homeostasis model assessment (HOMA-S). In the open-label phase, objectives included measurement of mean percentage change in BMD at the femoral neck as measured by DXA from weeks 52 to 76 within the RSG treatment group. The trial was approved by the relevant institutional ethical review boards, and all subjects signed informed consent.

Dual-energy x-ray absorptiometry

As detailed in a prior publication (40), areal BMD was measured by DXA instruments manufactured by Hologic, Inc. (Bedford, Massachusetts) or GE Healthcare Lunar (Madison, Wisconsin). Each subject had a minimum of 3 vertebral bodies evaluable at baseline, and all had DXA scans of the left femoral neck, total hip, and posterior-anterior L1–L4 lumbar spine. Standardized procedures for evaluating monthly instrument QC ensured stable instrument calibration, and cross-calibration of scanners was evaluated using Bona Fide Phantom (BioClinica Inc, Newtown, Pennsylvania). Subject DXA scans were performed at baseline and at weeks 16, 28, 52, and 76. All DXA scans were sent to a central reading facility for QC and analysis (BioClinica Inc), and all readers were blinded to group assignment.

Clinical laboratory measurements

Clinical laboratory assessments included: HbA1c (Ion-Exchange HPLC; Tosoh Bioscience, Inc., South San Francisco, California), fasting blood glucose (Olympus AU2700/5400; Olympus America, Inc., Center Valley, Pennsylvania), plasma insulin (Linco RIA; Linco Research, Inc, St Charles, Missouri) and serum IGF-I (Siemens DPC Immulite Chemiluminescence; Siemens Medical Solutions USA, Inc, Malvern, Pennsylvania), BSAP (Chemiluminescence Immunoassay/Beckman Access; Beckman Coulter, Inc., Brea, California), CTX (Electrochemiluminescence Immunoassay, Roche Elecsys; Roche Diagnostics, Inc, Indianapolis, Indiana), PINP radioimmunoassay (RIA) (Orion Diagnostica, Espoo, Finland), corrected serum calcium (Olympus AU2700/5400; calculated as [(4-serum albumin g/dL)*0.8 + serum calcium mg/dL]), 25-OHD (liquid chromatography tandem mass spectrometry), and PTH (DPC Immulite 2000 Intact PTH Assay). HOMA-S was determined as a percentage of values in a normal reference population using the HOMA calculator (41).

Statistical analyses

A computer-generated central randomization within each geographical region stratified patients by prior therapy (drug naive and prior monotherapy) and randomized them in a 1:1 ratio to 1 of 2 treatment arms. Sample size calculation was based on a 30% dropout rate and a SD of 4% for percentage change from baseline in femoral neck, ensuring that the 95% confidence interval will be the mean ± 0.9% for each treatment group. Treatment differences at 52 weeks for change from baseline and change from weeks 52 to 76 for selected parameters were assessed by an analysis of covariance with terms for treatment, baseline value, prior therapy, and region. The safety population, comprising all subjects who had received at least 1 dose of drug, was used for analysis of all parameters. The primary analysis was performed on the observed case dataset. In addition, supportive analyses were prespecified in the statistical analysis plan and performed based on last on-therapy observation. The supportive analyses (data not shown) yielded results that were consistent with the results obtained from the observed case analyses for the BMD parameters measured via DXA. The complete datasets were independently analyzed by the Clinical Trials Research Unit of the University of Sheffield and confirmed the data presented in this study.

Results

Demographics and baseline characteristics

Baseline characteristics have been discussed in detail previously (40), and a brief summary is provided here (Table 1). A total of 226 patients were randomized, with 225 receiving at least 1 dose of study medication (Figure 1). Patients entered either the RSG arm (n = 114) or the MET arm (n = 112). Patient demographic data were similar between the treatment groups at baseline. In the RSG arm, 77 patients completed 52 weeks, and 69 patients completed 76 weeks. In the MET arm, 85 completed 52 weeks, and 80 remained in the study at week 76.

Bone mineral density

Changes in BMD over time at the femoral neck, total hip, and lumbar spine are represented in Figure 2. At week 52, femoral neck mean BMD (± SE) by DXA decreased by −1.47 ± 0.52% with RSG compared with a +0.22 ± 0.51% increase with MET (treatment difference, 1.69%; $P = .01$; Figure 3A). The fall in femoral neck BMD with RSG at 52 weeks was attenuated when the subjects were

Table 1. Selected Baseline Characteristics

	RSG	MET	Total
n	114	111	225
Age, y	63.6 ± 6.61	64.0 ± 6.46	63.8 ± 6.52
Weight, kg	76.9 ± 16.03	76.9 ± 16.08	76.9 ± 16.06
BMI, kg/m^2	31.2 ± 5.86	31.5 ± 5.79	31.4 ± 5.82
Years postmenopausal	16.2 ± 7.79	17.7 ± 8.99	16.9 ± 8.39
Median duration of diabetes, y	3.9	3.3	3.6
HbA1c, %	6.8 ± 0.73	6.8 ± 0.74	6.8 ± 0.74
Femoral neck T-score	−0.96 ± 0.91	−0.97 ± 0.92	−0.97 ± 0.92
Total hip T-score	−0.07 ± 0.97	0.03 ± 0.97	−0.02 ± 0.97
Lumbar spine T-score	−0.57 ± 1.25	−0.52 ± 1.25	−0.55 ± 1.25

Abbreviation: BMI, body mass index. Data are expressed as mean ± SD, unless otherwise specified.

switched to MET. During the open-label MET phase, there was little change in femoral neck BMD in RSG- and MET-treated subjects (−0.07 vs −0.02%, respectively; $P = .94$; Figure 3A). From baseline to week 52, total hip BMD decreased with both RSG and MET treatment, with a greater loss in the RSG treatment group (−1.62 ± 0.39 vs −0.72 ± 0.38%, respectively; $P = .06$; Figure 3B). In contrast, BMD of the total hip increased from weeks 52 to 76 for patients previously given RSG, whereas a minimal nonsignificant decrease was observed in patients given MET throughout the study (+0.40 vs −0.13%, respectively; $P = .18$; Figure 3B). At week 52, RSG significantly reduced lumbar spine BMD compared with MET (−1.41 ± 0.42 vs +0.04 ± 0.42%; $P = .006$). Lumbar spine BMD increased for RSG- and MET-treated patients in the open-label MET phase (+0.26 vs +1.03%, respectively; $P = .18$; Figure 2C).

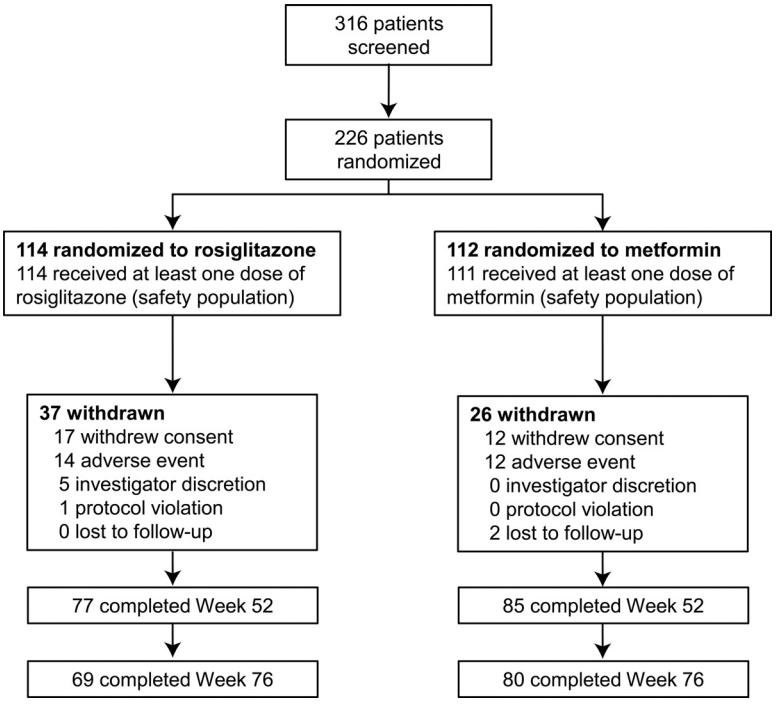


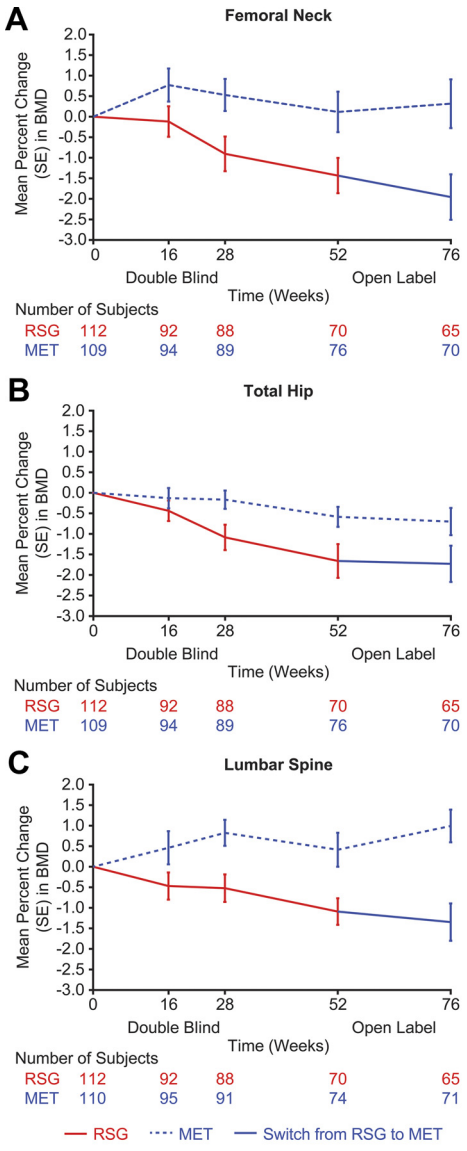
Figure 1. Patient disposition.

Bone turnover markers

From baseline to week 52, CTX (a marker of bone resorption) was significantly increased in the RSG group compared with the MET group (+18.1 vs −2.3%, respectively; $P = .012$; Figure 4A). During the open-label MET phase, the increase in CTX during the first 52 weeks of RSG exposure was completely reversed to or below baseline levels at week 76. The small decline in the MET group during the first 52 weeks was associated with an 8.4% gain at week 76. RSG was also associated with a significant increase in the bone formation marker PINP from baseline to week 52 compared with MET (+9.0 vs −13.3%, respectively; $P < .001$). As with CTX, the increase in PINP at week 52 was attenuated during the open-label MET extension. A significant reduction in PINP at week 76 was observed in patients previously treated with RSG compared with those only receiving MET (−12.4 vs +7.0%, respectively; $P < .001$; Figure 4B). Levels of the bone formation marker BSAP decreased in both treatment groups from baseline to week 52 (by −12.3% with RSG and −27.3% with MET; $P < .001$ for between-group comparison; Figure 4C). At week 76, a small decrease in BSAP was reported in patients on MET previously treated with RSG, and a significant increase was observed in patients treated only with MET (−2.0 and +8.0%, respectively; $P = .03$).

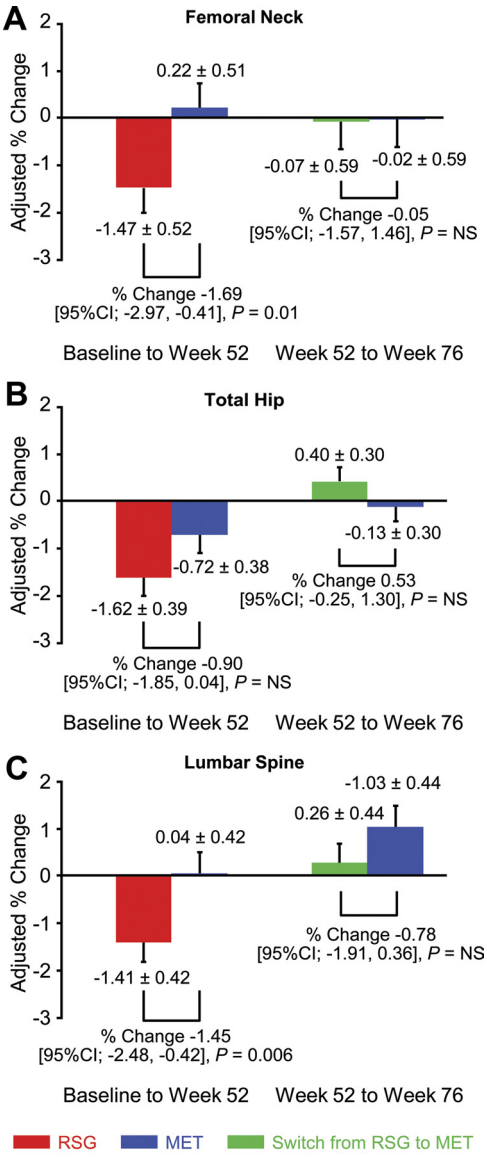
Parameters of calcium homeostasis and glycemic control

Albumin-adjusted serum calcium did not significantly change from baseline to week 52 in both treatment groups. Geometric means of serum 25-OHD levels were 73.1 and 73.9 nmol/L at baseline in the RSG and MET groups, respectively, and decreased in both groups from baseline to week 52, falling significantly more in the RSG treatment group compared with the MET group (−24.7 vs



BMD: bone mineral density; NS: nonsignificant; MET: metformin; RSG: rosiglitazone; SE: standard error

Figure 2. Mean percentage change in BMD over time from baseline for femoral neck (A), total hip (B), and lumbar spine (C) in subjects treated with RSG (solid red line) or MET (dotted blue line) for 52 weeks. At that time, subjects on MET were continued on MET, and subjects treated with RSG were crossed over to MET in an open-label continuation of the trial (solid blue line). *P* values for between-treatment group comparisons: femoral neck, baseline to week 52, $P = .01$; weeks 52 to 76, P = nonsignificant (NS); total hip, baseline to week 52, P = NS; weeks 52 to 76, P = NS; lumbar spine, baseline to week 52, $P = .006$; weeks 52 to 76, P = NS.

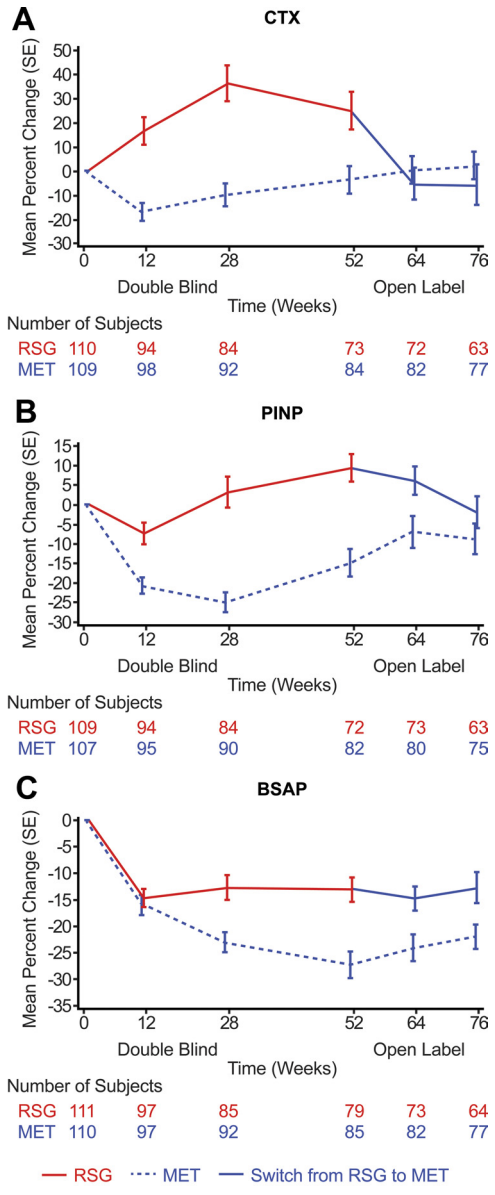


MET: metformin; RSG: rosiglitazone; NS: nonsignificant

Figure 3. Mean percentage change in BMD for femoral neck (A), total hip (B), and lumbar spine (C) during the double-blind phase of the study (RSG vs MET, baseline to wk 52) compared with the mean percentage change in BMD during the open-label MET phase (wk 52 to 76). *P* values reflect between-group comparisons. CI, confidence interval.

−12.2%; 14.2% difference between groups; $P = .004$). However, these differences were within the normal range (Table 2). Greater reductions in intact PTH were observed in the MET group compared with the RSG group from baseline to week 52 (RSG, −12.0%; MET, −22.0%; 12.8% treatment difference; $P = .07$), but these values were also within the normal range (Table 2).

Mean HbA1c decreased from baseline to week 52 by 0.5% in both the RSG and MET groups (Table 2). Reductions in HbA1c observed in both treatment groups from baseline to week 52 remained relatively stable to week 76. Reductions in FPG occurred with RSG and MET and increased in both treatment groups (+0.43 and +0.32 mmol/L for patients in the former RSG and MET groups, respectively) during the open-label phase. Fasting insulin levels increased in the MET treatment group (+7.5 pmol/L) but were decreased markedly in the RSG group (−11.5 pmol/L). Levels of fasting insulin increased in both groups from week 52 to week 76. At week 52, HOMA-S increased from baseline by +16.8% in the RSG group compared with a decrease of −5.2% in the MET group.



BSAP: bone-specific alkaline phosphatase; CTX: C-terminal telopeptides of type-I collagen; MET: metformin; PINP: procollagen type I N-terminal propeptide; RSG: rosiglitazone; SE: standard error

Figure 4. Mean percentage change in bone turnover markers CTX (A), PINP (B), or BSAP (C) after treatment with RSG or MET for 52 weeks, at which time all subjects were crossed over to open-label MET until week 76. *P* values for between-treatment comparisons: CTX, baseline to week 52, $P < .02$; weeks 52 to 76, $P < .001$; BSAP, baseline to week 52, $P < .001$; weeks 52 to 76, $P = .03$; PINP, baseline to week 52, $P < .001$; week 52 to 76, $P < .001$.

On change of administration of RSG to MET during the open-label phase, HOMA-S decreased by −16.7% and also continued to decrease in the MET-only treatment group (12.0%; Table 2). Changes in IGF-I values were unremarkable.

Safety

The incidence of on-therapy AEs was higher in the RSG group compared with MET (72 vs 65%, respectively, in the double-blind period; Table 3). This was due to a higher incidence of peripheral edema (11 vs 0%) and weight gain (8 vs <1%) in patients treated with RSG. The incidence of diarrhea was higher in the MET group (14 vs 3%). On-therapy AEs considered to be drug-related occurred in 30 and 24% of patients in the RSG and MET groups, respectively, from baseline to week 52 (Table 3). During the open-label phase, 9 and 2% of patients previously treated

Table 2. Calciotropic Hormones and Measures of Glycemic Control

	RSG	MET
Calciotropic hormones		
Calcium corrected (mmol/L), n	73/64/64	83/75/74
Baseline, mean (SD)	2.27 (0.086)	2.28 (0.074)
Week 52, mean (SD)	2.29 (0.068)	2.31 (0.086)
Week 76, mean (SD)	2.30 (0.068)	2.31 (0.082)
Vitamin D (nmol/L), n/n/n	61/55/63	65/58/76
Baseline geometric mean, CV (%)[a]	73.1 (41.62)	73.9 (39.20)
Week 52 geometric mean, CV (%)[a]	60.7 (40.15)	69.7 (37.45)
Week 76 geometric mean, CV (%)[a]	59.5 (34.07)	67.1 (32.76)
Intact PTH (ng/L), n/n/n	64/56/64	71/64/75
Baseline geometric mean, CV (%)[a]	36.4 (50.16)	39.3 (53.82)
Week 52 geometric mean, CV (%)[a]	31.5 (44.10)	31.5 (65.50)
Week 76 geometric mean, CV (%)[a]	29.6 (62.23)	32.1 (68.64)
Measures of glycemic control		
HbA1c (%), n/n/n	73/65/63	84/76/75
Baseline, mean (SD)	6.84 (0.728)	6.75 (0.740)
Week 52, mean (SD)	6.35 (0.741)	6.26 (0.811)
Week 76, mean (SD)	6.44 (0.782)	6.28 (0.672)
FPG (mmol/L), n/n/n	71/61/59	79/72/70
Baseline, mean (SD)	7.06 (1.746)	6.76 (1.505)
Week 52, mean (SD)	6.41 (1.644)	6.19 (1.313)
Week 76, mean (SD)	6.64 (1.23)	6.10 (1.28)
Fasting insulin (pmol/L), n/n/n	66/58/59	78/71/70
Baseline, mean (SD)	116.1 (78.80)	110.5 (65.57)
Week 52, mean (SD)	111.3 (90.51)	112.4 (65.79)
Week 76, mean (SD)	119.2 (58.60)	117.7 (64.91)
IGF-I (mmol/L), n/n/n	20/16/18	25/23/26
Baseline, mean (SD)	92.4 (38.39)	96.0 (31.68)
Week 52, mean (SD)	98.5 (38.77)	94.6 (32.01)
Week 76, mean (SD)	103.0 (41.81)	108.3 (46.31)
HOMA-S (%), n/n/n	69/59/59	75/71/68
Baseline geometric mean, CV (%)[a]	52.4 (48.94)	53.2 (48.57)
Week 52 geometric mean, CV (%)[a]	59.8 (52.43)	53.1 (45.22)
Week 76 geometric mean, CV (%)[a]	49.9 (46.47)	49.5 (37.34)

n/n/n represents the number of subjects with a baseline and week 52 value/number of subjects with a baseline and week 76 value/number of subjects with a week 52 and week 76 value.

[a] CV (coefficient of variation) = 100∗sqrt{exp{{SD on log scale}^2}−1}.

Table 3. Drug-Related on-Therapy AEs (≥2% in any Treatment Group)

	RSG, n (%)	MET, n (%)
n	114	111
Double-blind phase		
Any drug-related AE	34 (30)	27 (24)
Peripheral edema	12 (11)	0
Weight increased	9 (8)	1 (<1)
Dyspepsia	2 (2)	6 (5)
Back pain	2 (2)	1 (<1)
Fatigue	2 (2)	1 (<1)
Headache	2 (2)	0
Overweight	2 (2)	0
Diarrhea	1 (<1)	12 (11)
Nausea	1 (<1)	2 (2)
Open-label phase		
Any drug-related AE	10 (9)	2 (2)
Diarrhea	4 (4)	1 (<1)
Nausea	0	2 (2)

with RSG and MET, respectively, reported drug-related AEs. The incidence of AE withdrawals from the study was similar between treatment groups. No patient was withdrawn due to bone loss during the study.

The incidence of serious AEs (SAEs) was low and similar between the treatment groups (RSG, 6%; MET, 5%; Table 4). A single cardiac death (<1%) occurred in the RSG group in the double-blind period, which the investigator suspected to be treatment-related. There were 5 fractures in the RSG group and 1 fracture in the MET group during the double-blind phase of the study. In the RSG group, 1 patient experienced a fracture of the lumbar spine; she experienced back pain on study day 86 and was withdrawn from the trial. One patient on RSG fell in the street and fractured a lower leg (day 187); 2 patients fell from stairs and fractured a wrist (day 86) and fingers (day 27), respectively; and 1 patient inadvertently hit her toes against a wall while walking (day 181) and fractured her toes. The patient on MET fell and fractured her wrist (day 286). No SAEs or fractures were reported during the open-label period.

Table 4. Overview of SAEs and Other Significant AEs

	RSG, n (%)	MET, n (%)
n	114	111
Double-blind phase		
Deaths	1 (<1)	0
Any SAE	7 (6)	5 (5)
AEs leading to withdrawal from study	14 (12)	11 (10)
Any confirmed BMD loss	5 (4.4)	3 (2.7)
Fractures	5 (4)	1 (<1)
Hypoglycemia	16 (14)	16 (14)
Open-label phase		
Any SAE	0	0
AE leading to withdrawal from study	0	1 (<1)
Hypoglycemia	2 (2)	3 (3)

Discussion

In this study, we assessed the skeletal effects of RSG in postmenopausal women with T2DM without osteoporosis, a population at risk for fractures. The inclusion of MET, a first-line monotherapy or combination therapy used for the treatment of T2DM, provided an appropriate comparator treatment arm.

Treatment with RSG for 52 weeks was associated with small but significant reductions in BMD at the femoral neck as measured by DXA, with reductions similar in magnitude at the lumbar spine and total hip. These losses contrasted with small gains in BMD at the femoral neck and lumbar spine after 52 weeks of MET treatment and also were in contrast to smaller decreases in BMD at the total hip with MET. During the open-label period when all RSG patients were switched to MET, further bone loss was arrested at the femoral neck, and slight gains in BMD were seen at the total hip and lumbar spine.

The decreases in BMD were significantly greater with RSG than with MET, consistent with the observed increase in fracture risk with TZDs. Overall, these changes in BMD are small, and it is not clear whether the small changes fully account for the increased fracture risk with TZDs. Moreover, it is unknown whether longer term treatment with RSG is associated with the same rate of bone loss as that observed during the first year. It is possible that longer term treatment may result in cumulative bone loss that would be more detrimental to skeletal health than the changes seen in this 52-week study.

Increases in CTX and PINP with RSG treatment are suggestive of an increase in bone turnover. This is consistent with data from the studies by Gruntmanis et al (18) and Zinman et al (37) and provides a plausible explanation for the observed BMD loss. The results of the bone turnover marker BSAP are consistent with published data (30, 42) but discordant with the PINP results. One explanation could be that BSAP increases when mineralization processes are impaired. However, differences in the bone formation markers BSAP and PINP in this study are not completely understood.

It is notable that during the conduct of this trial there were many global changes to the regulatory label for RSG. There was the addition of language concerning an increased incidence of fracture that was observed in female patients taking RSG in a long-term trial. Additionally, warnings were added to the label regarding congestive

heart failure and ischemic heart disease, which limited the use of RSG worldwide. These changes could be responsible for the higher dropout rate in the RSG arm; however, this trial was double-blind, so the expectation is that dropouts would be similar between the 2 groups. It is notable that there are more patients removed from the trial due to investigator discretion in the RSG group compared to the MET group. However, this finding does not fully account for the difference in the dropout rate between the 2 groups.

The pathophysiology by which RSG causes bone loss and fracture remains elusive. Many hypotheses have been proposed, and it is likely that a combination of factors is responsible. BMD does not appear to explain the entire fracture risk in T2DM (6, 7) in that subjects with T2DM often have higher BMD than anticipated, given the increased risk of fracture. The small changes in BMD noted in this trial are unlikely to explain the increased fracture risk in this population. Other properties of bone in T2DM, such as microarchitecture, may play a role in assessing the fracture risk (43–45). T2DM as an independent risk factor has been proposed to be added to the FRAX algorithm (8). Although other nonskeletal aspects of T2DM may contribute to the risk of falling, eg, retinopathy, neuropathy, hypoglycemia and weight distribution, these factors in combination do not explain the increase in fracture risk in subjects with T2DM (46, 47).

TZDs are selective PPAR-γ agonists that alter gene transcription, leading to increased cellular insulin sensitivity in the liver and peripheral tissues. Activation of PPAR-γ by TZDs alters normal mesenchymal stem cell maturation to shift from an osteoblastic lineage to the adipogenic lineage (48). Although it is widely accepted that it is the shift in mesenchymal stem cell lineage that results in bone loss, new data have further explained this mechanism at a molecular level. In vitro data suggest that RSG-mediated activation and overexpression of PPAR-γ accelerates osteoblast differentiation (49). However, this process is followed by the accumulation of reactive oxygen species and apoptosis in the cells of the osteogenic lineage (49), which would result in a decrease in bone formation. In a recent publication, characteristics of circulating osteogenic precursors using flow cytometry revealed a lower percentage of positively labeled osteocalcin cells in T2DM (30). Molecular profiling indicated a decrease in Runx2, the major regulator of osteoblast formation (30). All of these mechanisms would result in inhibition of bone formation. The ensuing increase in adipose tissue may also contribute to the fracture risk in T2DM. Adipose tissue is a source of inflammatory cytokines and adipokines. These factors can increase bone resorption and/or decrease bone formation (22, 23). For example, adiponectin levels are associated negatively with BMD and positively with markers of bone resorption and vertebral fracture in select subjects with T2DM (24) and may also contribute to the pathophysiology of bone loss in T2DM.

The glycemic efficacy and safety findings for RSG in this study are consistent with other long-term studies of this agent (10, 50, 51). The population of postmenopausal women with T2DM had good glycemic control throughout the 52 weeks of the double-blind phase in both treatment groups, and mean HbA1c level was maintained below 6.5% after 1 year of treatment. Glycemic control was sustained during the open-label period. Daily treatment with either therapy during the 52-week initial study was generally well tolerated; open-label MET for 24 weeks also demonstrated good tolerability. The incidence of SAEs, AE withdrawals, and hypoglycemia was similar between treatment groups. Despite the glycemic control in this study, prolonged hyperglycemia has been implicated as a factor responsible for the increased fracture rate in T2DM. Changes in calciotropic hormones or growth factors have been proposed as a pathogenic mechanism for increased fracture risk in patients with T2DM (52, 53). It has been suggested that secondary hyperparathyroidism may be induced by increased urinary calcium (53). Alternatively, chronically decreased magnesium levels would also stimulate PTH. A recently published study in Japanese men and postmenopausal women with T2DM found that PTH levels were lower than in controls; this finding in conjunction with lower osteocalcin levels may result in bone of poorer quality (52). However, in this study no major perturbations were observed in calciotropic hormones.

Strengths of this study include: the randomized, double-blind design allowing comparison of 2 treatment types for 52 weeks; the 24-week open-label phase on MET providing the opportunity to assess whether the changes observed during the double-blind phase were reversible; and the simultaneous measurement of BMD and laboratory parameters of bone metabolism and turnover. DXA is a useful diagnostic tool but has limitations such as the ability to quantify changes in bone structure and composition. Additionally, the use of calcium and vitamin D may have attenuated the loss of bone, masking the full effect of RSG. The study was limited to postmenopausal women with BMD T-score > -2.5 and T2DM, which was selected as a vulnerable population that might be treated with TZDs. The selection of this study population limits the applicability of the results to a specific age range, gender, and hormonal status. The design does not allow us to determine whether the densitometric and dynamic effects on bone continue to progress with longer term dosing or whether they stabilize with time.

There have been several clinical trials to assess the effects of TZDs on bone turnover markers and BMD. Some of these studies have been small, of short duration, or in

nondiabetics (42, 54). This is the first study to collect prospective, randomized data from subjects with T2DM and include bone turnover markers, BMD, and calciotropic hormones. The study was designed to evaluate changes in femoral neck BMD from baseline in the RSG group. The femoral neck was chosen as the skeletal site of interest because it is a region measurable by DXA for which broadly accepted reference ranges are available. This site is comprised largely of cortical bone, and in other studies fractures in T2DM occurred at predominantly cortical sites in the appendicular skeleton. In addition, after 1 year of treatment, subjects were all treated with MET (open label), and BMD and bone turnover markers were measured 6 months later to assess reversibility of the effects of RSG. We noted a loss of BMD at the total hip and femoral neck that was partially attenuated during the open-label MET phase. The increases in CTX and PINP were consistent with an increase in bone turnover with RSG treatment and reverted toward baseline during the open-label MET phase of the study. No significant changes in calciotropic hormones explained these findings. This study is consistent with the published literature that the use of RSG is associated with increased bone turnover, bone loss, and an increased fracture risk in postmenopausal women with T2DM.

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