Effect of Rosiglitazone, Metformin, and Glyburide on Bone Biomarkers in Patients with Type 2 Diabetes

Bernard Zinman, Steven M. Haffner, William H. Herman, Rury R. Holman, John M. Lachin, Barbara G. Kravitz, Gitanjali Paul, Nigel P. Jones, R. Paul Aftring, Giancarlo Viberti, Steven E. Kahn, and the A Diabetes Outcome Progression Trial Study Group

Samuel Lunenfeld Research Institute (B.Z.), Mount Sinai Hospital and University of Toronto, Ontario, Canada MT5 3L9; University of Texas Health Science Center at San Antonio (S.M.H.), San Antonio, Texas 78229; Departments of Internal Medicine and Epidemiology (W.H.H.), University of Michigan, Ann Arbor, Michigan 48109; Diabetes Trials Unit (R.R.H.), Oxford Centre for Diabetes, Endocrinology, and Metabolism, Oxford OX3 7LJ, United Kingdom; Biostatistics Center (J.M.L.), George Washington University, Rockville, Maryland 20852; GlaxoSmithKline (B.G.K., G.P., R.P.A.), King of Prussia, Pennsylvania 19406; GlaxoSmithKline (N.P.J.), Harlow CM19 5AW, United Kingdom; King's College London School of Medicine (G.V.), London SE5 9RS, United Kingdom; and Division of Metabolism, Endocrinology, and Nutrition (S.E.K.), Department of Medicine, Veterans Affairs Puget Sound Health Care System and University of Washington, Seattle, Washington 98108

Context: An increase in bone fractures has been observed in women taking thiazolidinediones.

Objective: The objective of the study was to examine whether changes in circulating bone biomarkers provide insight into the underlying mechanisms responsible for the increase in bone fractures in female participants randomized to rosiglitazone in A Diabetes Outcome Progression Trial (ADOPT).

Research Design and Methods: Paired stored baseline and 12-month serum samples were available from 1605 participants (689 women, 916 men) in ADOPT, a long-term clinical trial comparing the effects of rosiglitazone, glyburide, and metformin on glycemic control in patients with type 2 diabetes.

Results: This subset was well matched to the total ADOPT study population. In women a marker of osteoclast activity, C-terminal telopeptide (for type 1 collagen), increased by 6.1% with rosiglitazone compared with reductions of 1.3% ($P = 0.03$ *vs.* rosiglitazone) and 3.3% ($P = 0.002$ *vs.* rosiglitazone) with metformin and glyburide, respectively. In men, C-terminal telopeptide was unchanged on rosiglitazone (−1.0%) and fell on metformin (−12.7%; $P < 0.001$) and glyburide (−4.3%, $P =$ NS). Markers of osteoblast activity, procollagen type 1 N-propeptide (P1NP) and bone alkaline phosphatase, were reduced for women and men in almost all treatment groups, with the greatest changes in the metformin group (P1NP in females, −14.4%; P1NP in males, −19.3%), intermediate for rosiglitazone (P1NP in females, −4.4%; P1NP in males, −14.4%), and smallest for glyburide (P1NP in males, +0.2%; bone alkaline phosphatase in females, −11.6%).

Conclusions: Commonly measured bone biomarkers suggest that changes in bone resorption may be partly responsible for the increased risk of fracture in women taking thiazolidinediones. **(*J Clin Endocrinol Metab* 95: 134–142, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-0572 Received March 16, 2009. Accepted October 2, 2009.
First Published Online October 29, 2009

Abbreviations: ADOPT, A Diabetes Outcome Progression Trial; CI, confidence interval; CTX, C-terminal telopeptide of type I collagen; P1NP, procollagen type I N-terminal propeptide.

Clinical evidence that thiazolidinediones increase the risk of fracture in women with type 2 diabetes was first demonstrated in A Diabetes Outcome Progression Trial (ADOPT) (1). ADOPT was a randomized, double-blind, prospective, controlled clinical trial comparing the effect of the thiazolidinedione rosiglitazone, biguanide metformin, and sulfonylurea glyburide on glucose control in recently diagnosed (<3 yr), drug-naïve patients with type 2 diabetes. Rosiglitazone was shown to result in more durable glycemic control compared with metformin or glyburide as assessed by fasting glucose and hemoglobin A1c. A review of adverse event reports found an increased occurrence of bone fractures in the upper and lower limbs, but not hip or vertebra, in women treated with rosiglitazone (1, 2). A corresponding increased incidence was not detected in men. After the publication of ADOPT, an increase in fracture risk in women treated with pioglitazone, the other thiazolidinedione in clinical use, was also reported (3), suggesting a possible class effect.

In vitro thiazolidinediones promote the differentiation of mesenchymal progenitor cells into adipocytes rather than osteoblasts (4, 5) and may suppress osteoblast formation by reducing IGF-1 levels in bone (6). Both rosiglitazone and pioglitazone have been shown to cause bone loss in rodents in most studies accompanied by decreased osteoblast activity and bone formation (7, 8) but in some by increased bone resorption (9, 10). Clinical studies suggest increased loss of bone mineral density in women treated with thiazolidinediones and bone biomarker data from three clinical trials support a hypothesis of reduced bone formation (11–13) without changes in resorption (13). However, these studies are limited by the small number of subjects and the short-treatment duration. A better understanding of the underlying pathophysiological mechanisms responsible for the observed thiazolidinedione-associated fractures is important because it may direct approaches to preventative treatment. We report the results of our analyses of serum bone metabolism biomarkers in a subset of participants in ADOPT.

Patients and Methods

Patients

The ADOPT study and details of its participants have been described previously (1, 14). In brief, eligible patients had type 2 diabetes diagnosed within 3 yr and were naïve to oral hypoglycemic drugs. They were 30–75 yr of age and had a fasting plasma glucose concentration between 126 and 180 mg/dl before randomization. Exclusion criteria were clinically significant liver disease, renal impairment, a history of lactic acidosis, unstable or severe angina, known heart failure requiring pharmacological intervention, uncontrolled hypertension, or chronic diseases requiring periodic or intermittent treatment with oral or iv corticosteroids, or continuous use of inhaled corticosteroids. Less than 2% of participants were on therapy for osteopenia or osteoporosis.

Protocol

The study was carried out in 488 centers in 17 countries in North America and Europe. A total of 4351 patients were randomly allocated to, and received double-blind treatment with rosiglitazone, metformin, or glyburide in a 1:1:1 ratio. The initial daily dose of rosiglitazone was 4 mg, metformin 500 mg, and glyburide 2.5 mg, and this was titrated to the maximum effective daily dose (rosiglitazone 4 mg twice daily, metformin 1 g twice daily, and glyburide 7.5 mg twice daily). Forced titration of the dose of medication occurred at each visit when the fasting plasma glucose level was 140 mg/dl or more. The primary outcome was time to monotherapy failure on maximum tolerated dose of the study drug, which was defined as a fasting plasma glucose greater than 180 mg/dl on two successive occasions or by independent adjudication (1).

The protocol was reviewed and approved by institutional review boards for each center, and all subjects gave written, informed consent. ADOPT was registered (clinicaltrials.gov, no. NCT00279045).

Reporting of fractures

Site investigators reported adverse events during the treatment portion of the study, and these were categorized using MedDRA (MedDRA dictionary, version 9.0; MSSO, Chantilly, VA). Fractures included any preferred term with the text fracture within the higher-level group term of bone and joint injuries. In the case of fractures, the site of the fractures was as reported to or determined by the investigators with no adjudication or subsequent directed assessment performed as part of the study protocol. No information on dietary calcium and vitamin D intake was obtained.

Laboratory analyses

In September 2000 the protocol and written informed consent were amended to include the drawing, storage, and poststudy analysis of serum samples taken in the fasting state in the morning both at baseline and after 12 months of treatment.

The bone minerals, calcium and phosphate, were measured by spectrophotometry using a modular autoanalyzer (Olympus America, Center Valley, PA), and the calcium-modulating hormones 25-hydroxyvitamin D by liquid chromatography with tandem mass spectrometry (Quest Nichols Institute Reference Laboratory, San Juan Capistrano, CA) and intact PTH by immunochemiluminescence (Siemens Medical Solutions, Culver City, CA). The bone resorption marker C-terminal telopeptide of type I collagen (CTX) was measured by ELISA (Nordic Biosciences, Copenhagen, Denmark), and the bone formation markers procollagen type I N-terminal propeptide (P1NP) and bone alkaline phosphatase by RIA (Orion Diagnostica, Espoo, Finland) and chemiluminescence (Beckman Access, Fullerton, CA), respectively. Estradiol was measured by RIA (Siemens Medical Solutions).

With the exception of calcium and phosphate that were measured real time during the course of the study, the other measurements were made as a single batch on baseline and 12-month samples that had been separated on site, shipped frozen, and then stored at −70 C before being assayed. All assays were performed at a

central laboratory (Quest, Van Nuys, CA), except CTX and 25-hydroxyvitamin D (Nichols Institute, San Juan Capistrano, CA).

Statistical methods

Data are presented as mean ± SD unless otherwise indicated. It was prespecified that analyses of CTX, P1NP, bone alkaline phosphatase, intact PTH, 25-hydroxyvitamin D, and estradiol, but not calcium and phosphate, would use the natural log to better approximate a normal distribution for errors and homoscedasticity of variances, as was verified by residual diagnostics. Changes or differences between groups for log-transformed variables were backtransformed to provide percent changes or percent differences in geometric means.

Analyses of the eight bone biomarkers were conducted separately among men and among women. Within each group the simple unadjusted change or percent change for a biomarker was computed, and the *P* value for the significance of the change was adjusted for three tests using Hochberg's method (15) separately among men and women. Differences between treatment groups in the change from baseline of a biomarker were assessed using an analysis of covariance model with terms for treatment group, gender, country, and the baseline biomarker measurement (16) separately among men and among women. Comparisons between rosiglitazone and glyburide and between rosiglitazone and metformin were tested. Within each gender, significance was assessed at the $P = 0.05$ level, two sided, adjusted for two treatment group comparisons using Hochberg's method (15) but were not adjusted for tests among the eight biomarkers in combination. Analyses were conducted using SAS (SAS Institute, Cary, NC).

Results

Demographic and clinical variables at baseline

Paired baseline and 12-month stored serum samples were available from 1605 patients (689 women, 916 men), representing 47% of ADOPT participants at 1 yr. This subset of 1605 patients was well matched to the total ADOPT study population (Table 1), there being no significant difference among groups. The average age of the bone biomarker cohort was 57 yr and among the women, 522 (76%) were postmenopausal by self-report. Those participating in the bone marker study differed from the remainder of the cohort within some groups for a few characteristics (Table 1) but not to a degree to influence the generalizability of the study results.

Time since diagnosis of diabetes, anthropomorphic measurements, such as body mass index, glycemic control at baseline, and the proportions of patients at baseline using selected categories of medications related to bone health did not differ among the treatment groups (Table 1). As anticipated, generally more women than men were using such medications (estrogen containing hormones, calcium supplements, bisphosphonates; data not shown).

Bone minerals, calcium-modulating hormones, and estrogen

The effect of 1 yr of treatment with rosiglitazone, metformin, and glyburide on these measures is listed in Table 2. In all groups, calcium levels changed by less than 0.04 mmol/liter and phosphate levels by less than 0.06 mmol/liter. Despite these differences being small, the changes in both minerals were significantly different in women receiving rosiglitazone compared with metformin.

Intact PTH fell in both the rosiglitazone (−17.2% women, −19.2% men) and metformin (−15.0% women, −14.1% men) groups, but was unaltered in patients receiving glyburide (+2.5% women, +2.9% men). 25-Hydroxyvitamin D increased in the metformin group (+10.9% women, +4.6% men), fell in the rosiglitazone group (−6.0% women, −2.0% men), and changed little in the glyburide group (+0.2% women, −2.5% men).

Levels of estradiol fell in the rosiglitazone group in both women and men. However, only in men was the difference in changes between rosiglitazone and metformin and rosiglitazone and glyburide statistically significant.

Changes in biomarkers of bone resorption and formation

The changes in bone biomarker levels from baseline to month 12 are given in Table 2. In women CTX, a marker of bone resorption, increased by 6.1% in the rosiglitazone group with little change in the metformin and glyburide groups (reductions of 1.3 and 3.3%, respectively). The difference in the response between rosiglitazone and the other two treatment groups was statistically significant. In men, by contrast, CTX was unchanged in the rosiglitazone group (−1.0%) and fell in the metformin (−12.7%; $P < 0.001$ for comparison with rosiglitazone) and glyburide groups (−4.3%, P = NS *vs.* rosiglitazone).

For the markers of bone formation, P1NP and bone alkaline phosphatase, reductions were seen for both women and men in almost all treatment groups, with the greatest changes in the metformin group (males: bone alkaline phosphatase, −16.4%, P1NP, −19.3%; females: bone alkaline phosphatase, −15.7%, P1NP, −14.4%); intermediate changes for rosiglitazone (males: bone alkaline phosphatase, −13.6%, P1NP, −14.4%; females: bone alkaline phosphatase, −12.6%, P1NP, −4.4%); and the smallest changes for glyburide (males: bone alkaline phosphatase, −6.8%, P1NP, +0.2% 14.4%; females: bone alkaline phosphatase, −11.6%, P1NP, −5.0%). The reductions in the metformin group in P1NP were significantly greater than those for rosiglitazone for both women and men. When comparing rosiglitazone with glyburide, only the reduction in P1NP in men was significantly different (−14.4% *vs.* +0.2%, respectively).

TABLE 1. Baseline demographic characteristics, clinical measures, and selected prior medication use in the bone biomarker substudy and the whole study cohort by treatment assignment

	Whole study cohort			Bone biomarker substudy		
	Rosiglitazone (n = 1456)	**Metformin (n = 1454)**	**Glyburide (n = 1441)**	**Rosiglitazone (n = 549)**	**Metformin (n = 551)**	**Glyburide (n = 505)**
Age (yr)	56.3 ± 10.0	56.9 ± 9.9	56.4 ± 10.2	56.9 ± 10.0	56.6 ± 9.4	56.7 ± 10.0
Women n (%)	645 (44.3)	590 (40.6)	605 (42.0)	250 (45.5)	225 (40.8)	214 (42.4)
Postmenopausal n, (percent of women)	498 (77.2)	463 (78.5)	449 (74.2)	193 (77.2)	176 (78.2)	153 (71.5)
Time since diagnosis of diabetes, n (%)						
<1 yr	650 (44.6)	673 (46.3)	637 (44.2)	242 (44.1)	247 (44.8)	223 (44.2)
1–2 yr	758 (52.1)	724 (49.8)	751 (52.1)	277 (50.5)	281 (51.0)	255 (50.5)
>2 yr	47 (3.2)	57 (3.9)	53 (3.7)	29 (5.3)	23 (4.2)	27 (5.3)
Body mass index (kg/m^2)	32.2 ± 6.7	32.1 ± 6.1[a]	32.2 ± 6.3	32.5 ± 6.5	32.8 ± 6.1	32.3 ± 6.7
Waist circumference (cm)	105.3 ± 14.6	105.6 ± 14.3[a]	105.6 ± 15.1	106.2 ± 14.7	106.9 ± 14.0	105.7 ± 15.5
Waist to hip ratio	0.95 ± 0.09	0.95 ± 0.10	0.94 ± 0.09	0.94 ± 0.08	0.95 ± 0.10	0.94 ± 0.09
Systolic BP (mm Hg)	133 ± 16	133 ± 15	133 ± 15	133 ± 16	133 ± 15	132 ± 14
Diastolic BP (mm Hg)	80 ± 9	80 ± 9	79 ± 9	80 ± 9	80 ± 9	79 ± 8
Fasting plasma glucose (mmol/liter) (mg/dl)	8.42 ± 1.43 (151.5 ± 25.8)	8.41 ± 1.42 (151.3 ± 25.6)	8.47 ± 1.52 (152.4 ± 27.3)	8.33 ± 1.29 (150.0 ± 23.3)	8.33 ± 1.32 (149.9 ± 23.7)	8.39 ± 1.41 (151.0 ± 25.3)
Hemoglobin A1c (%)	7.36 ± 0.93	7.36 ± 0.93	7.35 ± 0.92	7.36 ± 0.89	7.33 ± 0.83	7.37 ± 0.88
Fasting insulin (pmol/liter)	149.9 ± 108.2[a]	151.8 ± 111.6[a]	150.4 ± 113.1	160.0 ± 112.3	159.6 ± 114.2	154.3 ± 111.8
Estrogen containing hormones, n (%)	126 (8.7)	138 (9.5)	114 (7.9)	58 (10.6)	61 (11.1)	48 (9.5)
Calcium supplements, n (%)	52 (3.6)	67 (4.6)	45 (3.1)	23 (4.2)	28 (5.1)	20 (4.0)
Bisphosphonates, n (%)	13 (0.9)	13 (0.9)	9 (0.6)	3 (0.6)	6 (1.1)	4 (0.8)
Glucocorticoids, n (%)[b]	108 (7.4)	91 (6.3)	104 (7.2)	42 (7.7)	40 (7.3)	42 (8.3)
Glucocorticoids or Antiresorptive agents, n (%)	235 (16.1%)	229 (15.8%)	228 (15.8%)	101 (18.4%)	98 (17.8%)	95 (18.8%)
Thiazide diuretics, n (%)	229 (15.7)	219 (15.1)	220 (15.3)	95 (17.3)	82 (14.9)	77 (15.3)
Loop diuretics, n (%)	29 (2.0)	45 (3.1)	39 (2.7)	12 (2.2)	15 (2.7)	10 (2.0)

For all continuous variables, data are expressed as mean± SD. BP, Blood pressure.

[a] $P \leq 0.05$ for the difference between the subjects in the bone marker substudy *vs.* those not within a treatment group, adjusted for three tests separately for each characteristic. Within the bone marker cohort, the differences between rosiglitazone *vs.* metformin and glyburide were not significant for any characteristic, adjusted for two tests.

[b] Includes all routes of administration.

TABLE 2. Analyses of bone biomarkers: baseline and change at 12 months by gender and treatment group[a]

	Women			Men		
	Rosiglitazone (n = 250)	**Metformin (n = 225)**	**Glyburide (n = 214)**	**Rosiglitazone (n = 299)**	**Metformin (n = 326)**	**Glyburide (n = 291)**
Calcium (mmol/liter)						
Baseline mean ± SD	2.362 ± 0.100	2.352 ± 0.105	2.358 ± 0.102	2.356 ± 0.110	2.360 ± 0.087	2.360 ± 0.095
Mean change from baseline to 12 months (−SE, +SE)	−0.022 ± 0.006[b]	0.003 ± 0.007	−0.022 ± 0.007[c]	−0.020 ± 0.007[b]	−0.014 ± 0.005	−0.036 ± 0.005[c]
Rosiglitazone *vs.* control						
Adjusted mean difference		−0.020	0.001		−0.009	0.013
95% CI		−0.035, −0.005	−0.014, 0.017		−0.021, 0.004	0.000, 0.027
P value		0.009	NS		NS	NS
Phosphate (mmol/liter)						
Baseline mean ± SD	1.160 ± 0.150	1.140 ± 0.144	1.144 ± 0.153	1.056 ± 0.156	1.066 ± 0.160	1.088 ± 0.352
Mean change from baseline to 12 months (−SE, +SE)	0.010 ± 0.009	0.053 ± 0.010[b]	0.035 ± 0.012[c]	0.007 ± 0.008	0.023 ± 0.008	0.001 ± 0.021
Rosiglitazone *vs.* control						
Percent difference		−0.033	−0.018		−0.022	−0.159
95% CI		−0.057, −0.009	−0.042, 0.007		−0.045, 0.001	−0.0396, 0.008
P value		0.007	NS		NS	NS
Intact PTH (ng/liter)						
Baseline GM (CV, %)	35.9 (56.2)	35.8 (54.2)	36.2 (61.8)	33.6 (57.8)	37.0 (53.2)	33.7 (63.2)
Percent change from baseline at 12 months (−SE, +SE)	−17.2 (−19.9, −14.4)[b]	−15.0 (−17.8, −12.2)[b]	2.5 (−1.5, 6.6)	−19.2 (−21.6, −16.7)[c]	−14.1 (−16.4, −11.9)[b]	2.9 (−0.1, 5.9)
Rosiglitazone *vs.* control						
Percent difference		−2.2	−19.6		−8.9	−21.6
95% CI		−10.6, 6.9	−26.5, −12.0		−15.3, −2.0	−27.2, −15.5
P value		NS	<0.001		0.012	<0.001
25-Hydroxyvitamin D (nmol/liter)						
Baseline GM (CV, %)	55.7 (55.1)	53.0 (55.8)	54.7 (63.0)	63.4 (47.1)	62.9 (46.9)	60.5 (51.0)
Percent change from baseline at 12 months (−SE, +SE)	−6.0 (−8.0, −4.0)[b]	10.9 (8.4, 13.4)[b]	0.2 (−2.2, 2.7)	−2.0 (−3.5, −0.5)	4.6 (2.9, 6.4)	−2.5 (−4.2, −0.7)
Rosiglitazone *vs.* control						
Percent difference		−14.2	−5.7		−6.2	1.4
95% C.I.		−19.1, −8.9	−11.2, 0.1		−10.1, −2.0	−3.1, 6.0
P value		<0.001	NS		0.004	NS
Estradiol (pmol/liter)						
Baseline GM (CV, %)	164.4 (110.1)	155.4 (104.0)	151.5 (116.0)	105.8 (37.9)	104.6 (39.6)	108.6 (37.3)
Percent change from baseline at 12 months (−SE, +SE)	−15.4 (−20.3, −10.3)[b]	−7.1 (−12.2, −1.7)	−5.7 (−11.7, 0.8)	−9.1 (−11.0, −7.1)[b]	−1.2 (−3.0, 0.7)	−0.9 (−2.8, 1.1)
Rosiglitazone *vs.* control						
Percent difference		−6.5	−7.9		−7.6	−9.0
95% CI		−20.1, 9.4	−21.2, 7.7		−12.2, −2.8	−13.6, −4.2
P value		NS	NS		0.002	<0.001
CTX (ng/liter)						
Baseline GM (CV, %)	374 (45)	374 (45)	368 (45)	373 (38)	385 (43)	372 (41)
Percent change from baseline at 12 months (−SE, +SE)	6.1 (3.7, 8.7)	−1.3 (−3.8, 1.2)	−3.3 (−6.0, −0.6)	−1.0 (−3.0, 1.0)	−12.7 (−14.4, −10.9)[b]	−4.3 (−6.0, −2.5)
Rosiglitazone *vs.* control						
Percent difference		7.3	10.7		12.2	3.8
95% CI		0.7, 14.3	3.8, 18.1		(6.7, 18.0)	(−1.4, 9.2)
P value		0.029	0.002		<0.001	NS
P1NP (μg/liter)						
Baseline GM (CV, %)	32.3 (48.2)	33.6 (42.1)	33.2 (44.3)	33.3 (36.3)	34.6 (37.5)	33.0 (36.7)
Percent change from baseline at 12 months (−SE, +SE)	−4.4 (−6.2, −2.6)	−14.4 (−16.4, −12.4)[b]	−5.0 (−7.1, −2.8)	−14.4 (−15.9, −13.0)[c]	−19.3 (−20.7, −18.0)[c]	0.2 (−1.7, 2.1)
Rosiglitazone *vs.* control						
Percent difference		9.9	−0.1		4.9	−14.2
95% CI		4.0, 16.1	−5.5, 5.6		0.4, 9.7	−18.0, −10.2
P value		<0.001	NS		0.032	<0.001

(*Continued*)

TABLE 2. Continued

	Women			Men		
	Rosiglitazone (n = 250)	Metformin (n = 225)	Glyburide (n = 214)	Rosiglitazone (n = 299)	Metformin (n = 326)	Glyburide (n = 291)
Bone alkaline phosphatase (μg/liter)						
Baseline GM (CV, %)	12.7 (52.6)	12.9 (48.1)	12.3 (48.6)	12.5 (47.5)	11.6 (59.8)	12.3 (52.9)
Percent change from baseline at 12 months (−SE, +SE)	−12.6 (−15.3, −9.9)[b]	−15.7 (−17.8, −13.6)[b]	−11.6 (−14.7, −8.3)[c]	−13.6 (−15.8, −11.3)[c]	−16.4 (−18.9, −13.8)[c]	−6.8 (−9.4, −4.0)[b]
Rosiglitazone *vs.* control						
Percent difference		2.8	0.3		7.5	−5.9
95% CI		−4.6, 10.7	−7.0, 8.2		0.6, 15.0	−12.2, 0.7
P value		NS	NS		NS	NS

CV, Coefficient of variation; GM, geometric mean.

[a] For calcium and phosphate, baseline mean ± SD, unadjusted change within each group to 12 months, and baseline covariate adjusted difference between groups. For other variables analyzed using the log transformation, baseline geometric mean with coefficient of variation (percent) in *parentheses*, unadjusted percent change in the geometric mean within each group to 12 months, and adjusted percent difference in geometric means between groups. Separate models for men and women of each bone biomarker were adjusted for its baseline measurement and country. Separately among men and women, the *P* values for two treatment group comparisons were adjusted using Hochberg's method but were not adjusted for the analysis of eight different bone biomarkers. [b, c] *P* value for the change or percent change from baseline (not covariate adjusted) within each of the three treatment groups was adjusted for three tests using Hochberg's method, separately among men and women, and significance is designated as [b] $P \leq 0.01$; [c] 0.01 less than $P \leq 0.05$.

Comparisons within subsets of women

After eliminating the 483 women using glucocorticoids or antiresorptive agents at baseline or within the first 12 months, the results were largely unchanged except that the differences between rosiglitazone and either metformin or glyburide for CTX were diminished (2.1 and 6.2%, respectively) and no longer statistically significant.

The treatment group differences among the 167 pre- *vs.* the 522 postmenopausal women (*i.e.* the treatment by menopause status interactions) were significantly different for calcium but not any other markers. The level of calcium with rosiglitazone was 0.062 mmol/liter less than with metformin among premenopausal women [95% confidence interval (CI) 0.03, 0.094, nominal $P < 0.001$], whereas it was not significantly less among postmenopausal women (0.008 mmol/liter less, CI −0.009, 0.025, $P = 0.352$), the interaction $P = 0.003$ adjusted for two interaction tests. Likewise, the level with rosiglitazone was 0.055 mmol/liter less than with glyburide among premenopausal women (CI 0.025, 0.085, $P < 0.001$ *vs.* 0.022 mmol/liter greater among postmenopausal women (CI 0.004, 0.04 greater, $P = 0.018$), the interaction adjusted ($P < 0.001$). These differences among pre- and postmenopausal women persisted, although to a lesser degree, among the subset of women not taking glucocorticoids or antiresorptive agents.

Bone biomarkers and occurrence of fracture

A similar proportion of patients within the bone biomarker subset reported a fracture during the course of the study (82 of 1605, 5.1%) as for the whole study cohort (200 of 4351, 4.6%). The proportions by randomly assigned treatment groups were also similar between the bone biomarker subset (rosiglitazone, 34 of 549, 6.2%; metformin, 29 of 551, 5.3%; glyburide, 19 of 505, 3.8%) and the whole study population (rosiglitazone, 92 of 1456, 6.3%; metformin, 59 of 1454, 4.1%; glyburide, 49 of 1441, 3.4%).

The small magnitude of change in the measured bone biomarkers combined with the small number of patients reporting a fracture who also had biomarker data available to evaluate precluded formal analysis of a possible association between the two. However, as illustrated in Fig. 1 for CTX, P1NP, and bone alkaline phosphatase, there was no discernible difference in the pattern of bone biomarker levels at baseline or month 12 for those patients reporting a fracture during the study compared with that for the whole cohort.

Discussion

The measurement of bone biomarkers at baseline and at 1 yr in ADOPT does not provide a clear pathophysio-

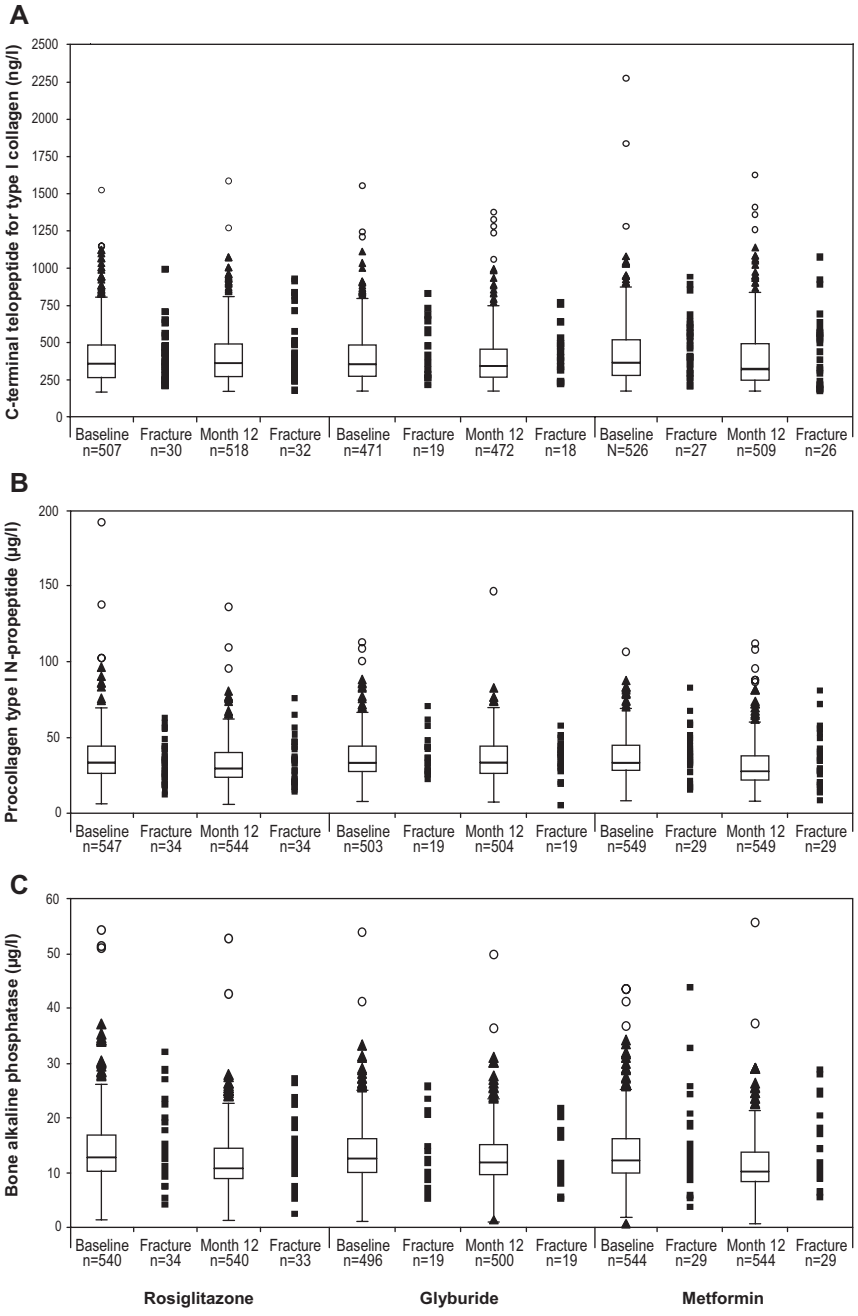

FIG. 1. Box and whisker plots and individual values for baseline and 12 months for CTX (A), P1NP (B), and bone alkaline phosphatase (C) for all patients and those with on-study fractures by treatment group. The box plot displays the 25th percentile, median, and 75th percentile as well as lower whisker [minimum observation above lower fence: 25th percentile − 1.5 × interquartile range (IQR)] and upper whisker (maximum observation below upper fence: 75th percentile + 1.5 × IQR). Extreme outliers (more than 3 times IQR from 25th/75th percentile) and mild outliers (more than 1.5 times but less than 3 times IQR from 25th/75th percentile) are also shown. Actual values of the parameters for patients experiencing fracture are displayed to the *right* of each box plot. To improve readability of the graph, some outliers in the metformin treatment were not plotted. In B, outlier values (248 µg/liter on metformin at baseline and 341 µg/liter on metformin at month 12) are not displayed on the graph. In C, outlier values (77.7 µg/liter on metformin at baseline and 93.9 µg/liter on metformin at month 12) are not displayed on the graph.

logical explanation for the observed increased risk of peripheral bone fractures in the women participants treated with rosiglitazone compared with those receiving metformin or glyburide. Changes in the osteoblast-associated markers of bone formation, P1NP and bone alkaline phosphatase, with rosiglitazone were intermediate between those with metformin and those with glyburide. In contrast, CTX, a biomarker of bone resorption, increased with rosiglitazone compared with both metformin and glyburide and is suggestive that increased bone resorption may have contributed to the increased fracture risk in the women treated with rosiglitazone. However, this is not definitive because the magnitude of the differences between the treatment groups, although statistically significant, is not large and may not be clinically relevant.

Of interest, *in vitro* and *in vivo* rodent studies suggest that the thiazolidinediones may reduce bone formation by preferentially directing the differentiation of mesenchymal progenitor cells to adipocyte lineage rather than to osteoblasts (4, 5, 7, 8). Furthermore, it has been suggested that these agents may also act to suppress osteoblast formation by reducing IGF-1 levels in bone (6). Although there are conflicting reports as to whether there may also be an effect mediated by increased bone resorption (7–10), the bone marker observations in ADOPT would support increased bone resorption with thiazolidinediones as a possibility.

Previous human studies demonstrated decreased bone mineral density with rosiglitazone in healthy postmenopausal women (13). In premenopausal women with polycystic ovary syndrome, investigators reported reduced bone mineral density associated with reductions in total alkaline phosphatase (12) and P1NP (13) with unchanged CTX levels (12, 13). However these were small studies (n per group: 25 and 15), and the duration of treatment was short (14 and 16 wk). The time frame in which these observation were made may be relevant because uncontrolled studies with troglitazone, a thiazolidinedione now withdrawn from clinical use, found that reductions in markers of both bone formation and resorption that were present at 1

month were no longer present after a year of therapy (17, 18). One further study, also with a small number of patients and of short duration reported reductions in bone alkaline phosphatase but no change in urine deoxypyridinoline, a marker of osteoclast activity, in diabetic patients treated with open-label rosiglitazone compared with controls managed by diet and exercise alone (11).

In addition to the much larger sample size of the cohort studied and the 12-month treatment period, our assessment of bone biomarkers in ADOPT has some advantages over these preceding studies. The population we studied is one for which the thiazolidinediones are clearly indicated. The biomarker cohort was drawn from a study in which the rosiglitazone-associated increase in fractures (as opposed to a surrogate such as bone mineral density) was observed. The presence of two separate, randomized, double-blind, control groups (metformin and glyburide), with similar fracture rates, reduces the chances of over interpreting single, nominally statistically significant findings when examining multiple analytes.

There clearly are limitations to the methodology we used. The study does not include a placebo arm, and by the time the protocol amendment governing these stored samples could be implemented, appreciable numbers of patients had already started randomized treatment. The samples were thus not collected truly systematically because the point from which sampling started was in large part dependent on the time for institutional approval in the 488 participating centers. Nonetheless, paired baseline and month 12 samples were collected for a large number of patients, representing nearly half of ADOPT participants at 1 yr. We believe this biomarker cohort is very likely to be representative of the ADOPT study as a whole because it was well matched with respect to baseline parameters and subsequent fracture occurrence with the overall ADOPT population. Our biomarker data are restricted to the first year of treatment in ADOPT, whereas the separation of fracture risk between rosiglitazone and controls became numerically distinct only after this time (2). However, whereas we made only a single on-treatment measurement and thus might have missed other changes in bone biomarkers that may have occurred before 12 months, precursor changes in the measured markers of bone metabolism would be expected to precede the clinical fracture events by a significant margin, making it quite unlikely that this substudy missed biomarker changes of importance by sampling too early. Finally, we did not quantify the effects of the different therapies on bone mineral density.

We had hoped that this analysis of commonly measured markers of bone metabolism drawn from participants in ADOPT would provide a clearer picture of the underlying pathophysiology responsible for the increase in bone fractures in women with type 2 diabetes treated with thiazolidinediones. Nonetheless, despite the absence of a placebo control group, the changes in bone markers with rosiglitazone do suggest an effect primarily on increased bone resorption without a major change in bone formation. Further controlled, long-term studies with measurement of bone markers, bone density, and clinical fractures rates will be required to demonstrate conclusively the underlying mechanisms responsible for thiazolidinediones-related bone fractures and the potential for prevention with various bone health interventions.

Acknowledgments

ADOPT was overseen by a steering committee comprised of Steven Kahn and Giancarlo Viberti (cochairs), Steven Haffner, William Herman, Rury Holman, Paul Aftring, Nigel Jones, John Lachin, and Bernard Zinman. The analyses presented here would not have been possible without the effort of the study participants and study staff. A list of members of the ADOPT Study Group appears elsewhere (N Engl J Med 2006;355:2427–2443).

Address all correspondence and requests for reprints to: Steven E. Kahn, M.B., Ch.B., Veterans Affairs Puget Sound Health Care System (151), 1660 South Columbian Way, Seattle, Washington 98108. E-mail: skahn@u.washington.edu.

This work was supported by funds from GlaxoSmithKline from whom the academic members of the ADOPT Steering Committee (B.Z., S.M.H., W.H.H., R.R.H., J.M.L., G.V., and S.E.K.) received honoraria, consulting fees, and/or grant/research support.

Disclosure Summary: R.P.A., G.P., B.G.K., and N.P.J. are employees of GlaxoSmithKline.

References

1. **Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G** 2006 Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med 355:2427–2443
2. **Kahn SE, Zinman B, Lachin JM, Haffner SM, Herman WH, Holman RR, Kravitz BG, Yu D, Heise MA, Aftring RP, Viberti G** 2008 Rosiglitazone-associated fractures in type 2 diabetes: an analysis from A Diabetes Outcome Progression Trial (ADOPT). Diabetes Care 31:845–851
3. **Takeda Pharmaceuticals** 2007 Observation of an increased incidence of fractures in female patients who received long-term treatment with ACTOS (pioglitazone HCl) tablets for type 2 diabetes mellitus (letter to health care providers)
4. **Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL** 2005 Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. Endocrinology 146: 1226–1235
5. **Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC, Jilka RL** 1999 Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARγ2. J Cell Biochem 74:357–371

6. **Lecka-Czernik B, Ackert-Bicknell C, Adamo ML, Marmolejos V, Churchill GA, Shockley KR, Reid IR, Grey A, Rosen CJ** 2007 Activation of peroxisome proliferator-activated receptor γ (PPARγ) by rosiglitazone suppresses components of the insulin-like growth factor regulatory system *in vitro* and *in vivo*. Endocrinology 148: 903–911
7. **Grey A** 2008 Skeletal consequences of thiazolidinedione therapy. Osteoporos Int 19:129–137
8. **Lecka-Czernik B, Suva LJ** 2006 Resolving the two "bony" faces of PPAR-γ. PPAR Res 2006:27489
9. **Lazarenko OP, Rzonca SO, Hogue WR, Swain FL, Suva LJ, Lecka-Czernik B** 2007 Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone. Endocrinology 148: 2669–2680
10. **Sottile V, Seuwen K, Kneissel M** 2004 Enhanced marrow adipogenesis and bone resorption in estrogen-deprived rats treated with the PPARγ agonist BRL49653 (rosiglitazone). Calcif Tissue Int 75: 329–337
11. **Berberoglu Z, Gursoy A, Bayraktar N, Yazici AC, Bascil Tutuncu N, Guvener Demirag N** 2007 Rosiglitazone decreases serum bone-specific alkaline phosphatase activity in postmenopausal diabetic women. J Clin Endocrinol Metab 92:3523–3530
12. **Glintborg D, Andersen M, Hagen C, Heickendorff L, Hermann AP** 2008 Association of pioglitazone treatment with decreased bone mineral density in obese premenopausal patients with polycystic ovary syndrome: a randomized, placebo-controlled trial. J Clin Endocrinol Metab 93:1696–1701
13. **Grey A, Bolland M, Gamble G, Wattie D, Horne A, Davidson J, Reid IR** 2007 The peroxisome proliferator-activated receptor-γ agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial. J Clin Endocrinol Metab 92:1305–1310
14. **Viberti G, Kahn SE, Greene DA, Herman WH, Zinman B, Holman RR, Haffner SM, Levy D, Lachin JM, Berry RA, Heise MA, Jones NP, Freed MI** 2002 A Diabetes Outcome Progression Trial (ADOPT): an international multicenter study of the comparative efficacy of rosiglitazone, glyburide, and metformin in recently diagnosed type 2 diabetes. Diabetes Care 25:1737–1743
15. **Hochberg Y** 1988 A sharper Bonferroni procedure for multiple tests of significance. Biometrika 75:800–802
16. **McCulloch CE, Searle SR** 2001 Generalized, linear and mixed models. New York: John Wiley, Sons
17. **Okazaki R, Miura M, Toriumi M, Taguchi M, Hirota Y, Fukumoto S, Fujita T, Tanaka K, Takeuchi A** 1999 Short-term treatment with troglitazone decreases bone turnover in patients with type 2 diabetes mellitus. Endocr J 46:795–801
18. **Watanabe S, Takeuchi Y, Fukumoto S, Fujita H, Nakano T, Fujita T** 2003 Decrease in serum leptin by troglitazone is associated with preventing bone loss in type 2 diabetic patients. J Bone Miner Metab 21:166–171