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Rosiglitazone Decreases Serum Bone-Specific Alkaline Phosphatase Activity in Postmenopausal Diabetic Women

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Objectives: Our objectives were to evaluate the effect of rosiglitazone on bone metabolism and to assess the association between changes in bone turnover parameters and plasma cytokine levels in postmenopausal diabetic women.

Design: This was a 12-wk open-label randomized-controlled trial.

Patients or Other Participants: A total of 56 obese postmenopausal women with newly diagnosed diabetes and 26 nondiabetic healthy controls matched for age and body mass index were included in the study.

Interventions: The subjects were instructed to follow a weight-maintenance diet. Half were randomly assigned to receive rosiglitazone 4 mg/d, and the other half remained on diet alone.

Main Outcome Measures: Before and after the interventions, metabolic bone markers and serum cytokine levels were assessed.

Results: Serum total alkaline phosphatase (ALP) and bone-specific ALP levels were statistically significantly lower 12 wk after initiation of rosiglitazone treatment. There were no statistically significant changes in osteocalcin levels among the three groups or in deoxypyridinoline levels in the rosiglitazone group. At the end of 12 wk, all patients had statistically significantly decreased IL-1β and TNF-α levels compared with baseline. Changes in bone-specific ALP levels showed a moderate negative correlation with the changes in the TNF-α levels after rosiglitazone treatment and after diet in the diabetic control group.

Conclusions: Rosiglitazone use is associated with reduced bone formation at earlier stages in postmenopausal diabetic women. The cytokine-lowering effects of rosiglitazone and lifestyle changes could reverse the early inhibitory effect of rosiglitazone therapy on bone formation. Further studies will clarify the long-term effects of rosiglitazone therapy on bone loss and fracture. **(*J Clin Endocrinol Metab* 92: 3523–3530, 2007)**

OBESITY AND TYPE 2 diabetes are states of low-grade systemic inflammation (1). Plasma concentrations of TNF-α and IL-6 are elevated in obese individuals and in those with type 2 diabetes (2–4). In addition, cytokines are thought to play a role in the pathogenesis of postmenopausal osteoporosis (5, 6). Estrogen deficiency in postmenopausal women induces bone resorption, at least in part, by mediating an enhanced paracrine production of cytokines (7). An elevated TNF-α level has been identified as an early and significant contributor to osteoclastogenesis. The consequent secretion of IL-1β and IL-6 further exacerbates the destruction of bone (8). The increased production of haptoglobin in obese humans may also contribute to bone loss by inducing the formation of prostanoids that enhance bone resorption (9, 10). Thiazolidinediones (TZDs) act as ligands for peroxisome proliferator-activated receptor-γ (PPAR-γ), which directly regulates genes involved in glucose homeostasis and adipogenesis (11). PPAR-γ has also been implicated in inflammatory responses (12). PPAR-γ2 isoform appears to play a crucial role in the process of osteoblast and adipocyte differentiation from common bone marrow mesenchymal stem cells. PPAR-γ2 activation by TZDs may affect bone through an increase in bone adiposity at the expense of the number and differentiation capacity of osteoblast progenitors, which results in reduced bone formation (13). Simultaneously, TZDs may inhibit osteoclastic bone resorption through their direct local effects on bone marrow cells (14). On the contrary, the decline in serum leptin without decrease in body fat mass by TZDs may be involved in preventing bone loss (15).

TZDs have caused bone loss in some (13, 16, 17), but not all (18), subjects in rodent studies. To date, research that specifically addresses the effects of TZD administration on human bone has been limited. Administration of TZDs reduced markers of bone turnover and is associated with detrimental skeletal effects and a higher incidence of fractures in several human studies (15, 19–22).

Whether TZDs have a beneficial or detrimental effect on human bone remains unclear. In addition, the correlation between cytokine levels and bone turnover parameters in postmenopausal diabetic women receiving TZD therapy has not, to our knowledge, been previously investigated. We hypothesized that the cytokine-lowering effect of TZDs may play a beneficial role in bone metabolism. For that reason, we conducted this open-label randomized-controlled trial to

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Abbreviations: ALP, Alkaline phosphatase; BMI, body mass index; bsALP, bone-specific ALP; Ca, calcium; Cr, creatinine; DPD, deoxypyridinoline; HbA1c, glycosylated hemoglobin; HOMA, homeostasis model assessment; OCL, osteocalcin; PO_4, phosphate; PPAR-γ, peroxisome proliferator-activated receptor-γ; TZD, thiazolidinedione.

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evaluate the effect of rosiglitazone on bone turnover parameters. We also aimed to assess the association between the changes in bone turnover parameters and plasma levels of IL-1β, IL-6, TNF-α, and haptoglobin.

Patients and Methods

The Baskent University Human Research Ethics Committee approved the study protocol. All participants provided written informed consent before study enrollment.

Patients

The recruitment period began in June 2005 and was completed at the end of October 2005. Subjects with an increased risk for diabetes (*i.e.* a family history of diabetes or a history of gestational diabetes, obesity, hypertension, and/or dyslipidemia) were selected. The subjects eligible for our study were postmenopausal women whose last menses was at least 2 yr before initiation of the study and who had a FSH level more than 40 IU/liter. Those individuals were invited to undergo a standard oral glucose tolerance test (glucose load, 75 g). For definitive inclusion, the 2-h post-load glucose level had to be ≥200 mg/dl, with or without a fasting plasma glucose level ≥126 mg/dl and with a fasting C-peptide level more than 1.0 ng/ml.

The exclusion criteria were: patients with a recent fracture or osteoporosis; treatment with drugs that might affect calcium (Ca) or bone metabolism (*e.g.* estrogens, calcitonin, bisphosphonates, anabolic steroids, raloxifene, Ca supplements with vitamin D, thyroxine, lithium, and drugs known to interfere with cytokine release, such as corticosteroids, immunosuppressors, or nonsteroidal antiinflammatory drugs); a thyroid, parathyroid, pituitary, nutritional, inflammatory, hepatic, renal, or neoplastic disorder; or severe cardiovascular disease (*e.g.* New York Heart Association class III or IV congestive heart failure or a history of myocardial infarction or stroke).

Of the 95 subjects participating in the screening oral glucose tolerance test, 61 had type 2 diabetes mellitus. Five of those diabetic women met the exclusion criteria: one was undergoing treatment with a bisphosphonate, one was being treated with glucocorticoid, one had primary hyperparathyroidism, and two had osteoporosis. The remaining 56 diabetic patients were enrolled in and completed the study. A total of 26 healthy subjects matched for age and body mass index (BMI) served as nondiabetic controls. No power calculation was performed to determine the sample size. Instead, the sample size was chosen based on the study feasibility.

All subjects were instructed to follow throughout the study a weight-maintaining diet based on recommendations from the American Diabetes Association (23). All subjects were also encouraged to walk or jog for at least 30 min daily. Of the diabetic subjects, 28 were randomly assigned to receive rosiglitazone (4 mg/d) in accordance with the two-factor design with repeated measures on one factor. Twenty-eight of the diabetic subjects were studied on diet alone. The allocation sequence was generated independent of the research team. Allocations were placed in sequentially numbered sealed opaque envelopes. Staff working in the department used the next envelope in the sequence to allocate participants.

The treatment period lasted 12 wk. All subjects worked with a clinical research dietitian every 4 wk. The dietary Ca intake determined via a food-frequency questionnaire remained as usual throughout the study. Compliance was tested by pill counts in the rosiglitazone group, but in diabetic and nondiabetic subjects on the prescriptive diet only, weight maintenance was considered a marker of compliance.

Methods

All subjects underwent a complete clinical examination, anthropometric measurements, and laboratory tests at baseline, and at the end of the 12th week of the study. Body fat measurements that were used to determine the body fat percentage and the total body fat mass were obtained in fasting subjects by a leg-to-leg bioelectric impedance device (TBF-300 M; Tanita Corp., Tokyo, Japan). All measurements were obtained by the same investigator.

Laboratory investigations included the assessment of glycemic control [levels of glycosylated hemoglobin (HbA1c), fasting plasma glucose, and insulin, and the homeostasis model assessment (HOMA) index] (24), levels of serum bone-specific alkaline phosphatase (bsALP), and the active human osteocalcin (OCL) concentration as markers of bone formation, and the level of urine deoxypyridinoline (DPD) as a marker of bone resorption. Other nonspecific bone markers, including serum total alkaline phosphatase (ALP) activity and urinary Ca and phosphate (PO_4) concentrations, were also measured. Urine concentrations of DPD (nmol/liter), Ca (mmol/liter), and PO_4 (mmol/liter) were corrected for their respective urine creatinine (Cr) concentrations in mmol/liter (urine DPD/Cr, urine Ca/Cr, and urine PO_4/Cr, respectively). In addition, fasting blood samples were analyzed for plasma cytokine levels, including levels of IL-1β, IL-6, and TNF-α, and for the haptoglobin level.

Laboratory analysis

After a minimum 8-h overnight fast, venous blood samples were drawn between 0800 and 0900 h after the patients had experienced approximately 30 min of supine rest. The first morning urine sample was collected without a preservative before 1000 h to avoid any possible effect from diurnal variation on the DPD. All blood samples were centrifuged with sera aliquoted immediately after collection. They were deep frozen and stored at −70 C with all the urine samples until the samples were analyzed in a single batch.

The HbA1c level was measured with HPLC (DIAMAT; Bio-Rad Laboratories, Milan, Italy). Serum glucose was assayed with a hexokinase method (Roche Diagnostics, Mannheim, Germany). The serum insulin level was assayed with a solid-phase competitive chemiluminescent enzyme immunoassay (Diagnostic Product Corp., Los Angeles, CA).

TABLE 1. Demographic and anthropometric characteristics before and after interventions

Parameter	Rosiglitazone $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Diabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Nondiabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 26)		
	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value
Age (yr)	59.7 ± 1.2			59.8 ± 1.6			58.4 ± 1.2		NS
Duration of menopause (yr)	11.7 ± 1.3			11.8 ± 1.7			11.8 ± 1.2		NS
BMI (kg/m^2)	34.6 ± 0.8 *34.6* (30–44)	35.9 ± 0.9 *36* (30–45.1)	<0.001	33.7 ± 0.6 *33.3* (30.2–40.0)	33.9 ± 0.6 *33.7* (30–41.3)	NS	34.6 ± 1.7 *33.6* (30–46.29	34.4 ± 1.0 *33.7* (30–46.7)	NS
Waist circumference (cm)	102.0 ± 1.8	103.4 ± 1.7	<0.05	102.6 ± 1.8	102.8 ± 1.7	NS	102.3 ± 1.9	102.3 ± 1.8	NS
Body fat (%)	41.1 ± 0.8	42.1 ± 0.9	<0.05	40.3 ± 0.8	40.4 ± 0.9	NS	41.0 ± 0.9	41.3 ± 0.9	NS
Body fat (kg)	34.6 ± 1.4	35.6 ± 1.4[a]	<0.001	34.4 ± 1.4	34.6 ± 1.4	NS	35.4 ± 1.4	36.0 ± 1.5	NS

The results of nonparametric tests were expressed as the number of observations (n), the mean ± SEM, and the median and minimum-maximum values [$\bar{X} \pm S_{\bar{X}}$, M (min-max)]. There were no differences in baseline values between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

[a] $P < 0.05$ effect of the interventions.

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TABLE 2. Statistical analysis of the clinical characteristics of the study subjects before and after interventions

Parameter	Rosiglitazone $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Diabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Nondiabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 26)		
	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value
Fasting glucose (mg/dl)	125.2 ± 3.9 *123* (92–177)	110.6 ± 3.1 *107* (91–155)	<0.001	118.6 ± 3.3 *115.5* (96–165)	113.7 ± 2.6 *111* (90–139)	NS	94 ± 1.1 *95.5* (77–99)	93.4 ± 1.0 *95* (81–99)	NS
Fasting insulin (μIU/ml)	16.9 ± 2.8 *12.2* (4.1–74.7)	10.9 ± 1.9 *9.8* (0.5–51.0)	<0.05	12.4 ± 1.4 *10.0* (3.1–35.5)	10.1 ± 0.9 *8.8* (3.7–23.0)	NS	10.3 ± 0.9 *9.6* (2.6–23.0)	11.1 ± 1.6 *8.7* (4.3–44.3)	NS
HOMA index	5.3 ± 0.9 *3.4* (1.2–23.2)	3.5 ± 0.7 *2.6* (0.1–19.5)	<0.01	4.7 ± 0.5 *3.2* (1.2–13.4)	3.8 ± 0.2 *2.3* (1.0–5.9)	NS	2.4 ± 0.2 *2.3* (0.6–5.2)	2.6 ± 0.4 *2.0* (0.9–10.4)	NS
HbA1c (%)	6.34 ± 0.1	6.18 ± 0.1	NS	5.96 ± 0.1	6.1 ± 0.1	NS	4.54 ± 0.1	4.53 ± 0.1	NS
Urine Ca/Cr	0.3 ± 0.3	0.3 ± 0.3	NS	0.3 ± 0.5	0.3 ± 0.2	NS	0.3 ± 0.3	0.3 ± 0.3	NS
Urine PO_4/Cr	1.7 ± 1.0	2.0 ± 0.9	NS	1.8 ± 0.9	1.9 ± 0.8	NS	1.8 ± 0.9	1.8 ± 0.9	NS

The results of nonparametric tests were expressed as the number of observations (n), the mean ± SEM, and the median and minimum-maximum values [$\bar{X} \pm S_{\bar{X}}$, M (min-max)]. Baseline values were not statistically significantly different between the groups except with respect to the fasting glucose level, HOMA index, and HbA1c level, all of which were lowest in the nondiabetic control group ($P < 0.001$, $P < 0.01$, and $P \leq 0.01$, respectively). M, Median; max, maximum; min, minimum; NS, not significant.

Serum bsALP assays were performed with an enzyme immunoassay method (Metra BAP EIA kit; Quidel Corp., San Diego, CA).

Levels of serum IL-1β, IL-6, and TNF-α were assayed with a commercially available ELISA test kit (BioSource Intl., Fleurus, Belgium). The detection limits of IL-1β, IL-6, and TNF-α were less than 1, less than 2, and less than 2 pg/ml, respectively.

Serum OCL assays were performed with a solid-phase two-site chemiluminescent immunometric assay method (Diagnostic Product Corp.). The level of urine DPD was measured via a solid-phase chemiluminescent enzyme-labeled immunoassay (Diagnostic Product Corp.). The serum haptoglobin level was assayed via an immunoturbidimetric assay with a Hitachi Modular PP analyzer (Roche Diagnostics) according to the manufacturer's instructions.

Statistical analysis

Homogeneities of data sets were controlled by the Levene test. The normality of distribution of variables was controlled by the Shapiro-Wilk test. Normally distributed variables were compared with the one-way ANOVA, after which multiple comparisons between pairs of groups were conducted according to the Duncan test. The two-factor repeated measures ANOVA was used to compare the groups at baseline and at the end of the study period. The Bonferroni test was then used for multiple comparisons. The results are expressed as the number of observations and the mean ± SE of the mean ($\bar{X} \pm S_{\bar{X}}$). Parametric test assumptions were not available for some variables, so the comparisons of those variables were performed with the Kruskal-Wallis and Wilcoxon signed rank tests. The Kruskal-Wallis test was followed by the Dunn test for multiple comparisons. The results of nonparametric tests were expressed as the number of observations, the mean ± SE of the mean, and the median and minimum-maximum values [$\bar{X} \pm S_{\bar{X}}$, M(min-max)]. The Pearson product-moment correlation coefficient was used to show the relationships between normally distributed variables. The Spearman's rank correlation coefficient was used to evaluate the correlations between nonnormally distributed variables.

Data analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0; SPSS, Inc., Chicago, IL). A *P* value less than 0.05 was considered statistically significant.

Results

All groups were matched for age, BMI, body composition, and clinical characteristics at baseline (Tables 1-4).

Changes in anthropometric and clinical characteristics after interventions at 12 wk

As shown in Table 1, the changes in BMI and body composition were more marked in the rosiglitazone group; however, the only statistically significant between-group difference was in total body fat mass. The increase in the mean BMI after rosiglitazone treatment was attributed to an increase in fat mass (1.9 kg).

The mean changes in clinical characteristics are shown in Table 2. When compared with the baseline value, the mean

TABLE 3. Statistical analysis of nonspecific and specific bone markers before and after interventions

Parameter	Rosiglitazone $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Diabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Nondiabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 26)		
	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value
Total ALP (IU/liter)	82.7 ± 4.4	69.2 ± 4.1[a]	<0.001	79.2 ± 4.4	73.3 ± 4.1	NS	84.8 ± 4.6	84.4 ± 4.2	NS
bsALP (IU/liter)	28.9 ± 2.1	22.7 ± 2.0[a]	<0.01	29.4 ± 2.1	28.2 ± 2.0	NS	31.6 ± 2.1	30.1 ± 2.0	NS
OCL (ng/ml)	3.5 ± 0.5 *3.3* (1.0–10.5)	2.8 ± 0.4 *1.9* (1.0–9.0)	NS	3.4 ± 0.7 *3.5* (1.0–13.7)	2.8 ± 0.3 *1.8* (1.0–8.3)	NS	3.6 ± 0.4 *2.9* (1.0–9.9)	2.9 ± 0.4 *2.3* (1.0–8.3)	NS
Urine DPD (nmol/Cr)	10.2 ± 0.9	10.5 ± 0.8	NS	10.2 ± 0.9	11.9 ± 0.9	<0.01	9.2 ± 0.5	10.8 ± 0.8	<0.01

The results of nonparametric tests were expressed as the number of observations (n), the mean ± SEM, and the median and minimum-maximum values [$\bar{X} \pm S_{\bar{X}}$, M (min-max)]. The baseline values were not significantly different between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

[a] $P < 0.05$ effect of the interventions.

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TABLE 4. Statistical analysis of cytokine and haptoglobin concentrations before and after interventions

Parameter	Rosiglitazone $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Diabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 28)			Nondiabetic control $\bar{X} \pm S_{\bar{X}}$ M (min-max) (n = 26)		
	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value	Baseline	12th wk	*P* value
IL-1β (pg/ml)	0.23 ± 0.10 *0.03* (0.0–2.7)	0.11 ± 0.10 *0.004* (0.0–2.5)	<0.001	0.21 ± 0.12 *0.05* (0.0–3.3)	0.14 ± 0.12 *0.003* (0.0–0.5)	<0.05	0.23 ± 0.18 *0.02* (0.0–4.8)	0.12 ± 0.10 *0.004* (0.0–0.009)	<0.01
IL-6 (pg/ml)	3.5 ± 0.6 *3.1* (0.9–15.6)	2.9 ± 0.5 *2.2* (0.5–11.5)	NS	2.8 ± 0.3 *2.5* (0.8–6.3)	2.9 ± 0.4 *2.4* (0.8–8.8)	NS	3.2 ± 0.4 *3.0* (1.1–11.4)	2.7 ± 0.4 *1.7* (0.6–7.2)	NS
TNF-α (pg/ml)	13.9 ± 0.6 *13.6* (10.3–24.6)	9.8 ± 0.4 *9.2* (7.7–19.9)	<0.001	13.6 ± 0.8 *13.6* (10.0–25.8)	9.6 ± 0.3 *9.4* (7.3–14.3)	<0.001	13.0 ± 0.7 *12.2* (7.6–24.0)	10.1 ± 0.6 *9.5* (6.5–17.9)	<0.001
Haptoglobin (mg/dl)	139.2 ± 8.8	141.8 ± 8.4	NS	136.7 ± 8.8	138.0 ± 8.4	NS	132.5 ± 9.1	141.6 ± 8.7	NS

The results of nonparametric tests were expressed as the number of observations (n), the mean ± SEM, and the median and minimum-maximum values [$\bar{X} \pm S_{\bar{X}}$, M (min-max)]. The baseline values were not significantly different between the groups. M, Median; max, maximum; min, minimum; NS, not significant.

fasting plasma glucose level had decreased in the rosiglitazone group ($P < 0.001$). Furthermore, when compared with the baseline values, a statistically significant decrease in the mean fasting plasma insulin level and the HOMA index was observed in the rosiglitazone group ($P < 0.05$ and $P < 0.01$, respectively). The HbA1c level did not change with statistical significance in the three groups. However, the difference between the mean changes in the HbA1c level in the rosiglitazone and the diabetic control groups approached statistical significance. Serum total ALP and bsALP levels (Fig. 1) were markedly lower in the rosiglitazone group ($P < 0.001$ and $P < 0.01$, respectively). The bsALP level decreased by 21% after rosiglitazone treatment. However, the values of those parameters did not change in the other two groups. The differences in the changes in those parameters among the rosiglitazone group and other two groups were statistically significant ($P < 0.05$ for both parameters). Although the changes were not statistically significant, the OCL levels decreased by 20, 18, and 14% in the rosiglitazone, diabetic control, and nondiabetic control groups, respectively. The DPD level showed statistically significantly higher urine concentrations at 12 wk than at baseline in the control groups. No statistically significant change was observed in the DPD level in response to rosiglitazone treatment. However, there was no statistically significant between-group difference.

At the end of 12 wk, patients in the three groups demonstrated statistically significantly decreased levels of IL-1β and TNF-α compared with baseline ($P = 0.001$ and $P < 0.001$, respectively) (Table 3). No statistically significant changes or differences were observed in the IL-6 and haptoglobin levels in the three groups.

Baseline correlations (Table 5)

Among the metabolic bone markers, urinary DPD and serum OCL concentrations did not correlate with serum bsALP levels at baseline. In all subjects, baseline total ALP concentrations were positively correlated with IL-6 levels. Baseline bsALP concentrations were negatively correlated with TNF-α levels (Fig. 2). In addition, there was a negative correlation between the bsALP level and the duration after menopause. OCL concentrations were inversely correlated with total body fat, the HOMA index, HbA1c level, and IL-6 level.

However, the correlations among OCL concentrations and plasma TNF-α and IL-1β concentrations did not reach statistical significance. The urinary DPD level did not correlate with cytokines. The baseline IL-6 level correlated with the IL-1β level but not with the TNF-α level.

Correlations after interventions (Table 6)

For the rosiglitazone-treated group, the changes in the total ALP concentration were not significantly correlated with changes in the TNF-α and IL-1β levels. In contrast, the changes in total ALP concentrations were positively correlated with changes in the HOMA index and IL-6 levels. The changes in the bsALP levels showed a moderate negative correlation with the changes in the TNF-α levels after rosiglitazone therapy (Fig. 3). The correlations between bsALP concentrations and changes in the levels of IL-1β and IL-6 were weak and not statistically significant.

In the diabetic control group, the changes in bsALP levels showed a negative correlation with the changes in the TNF-α and HbA1c levels. The changes in OCL levels showed a mod-

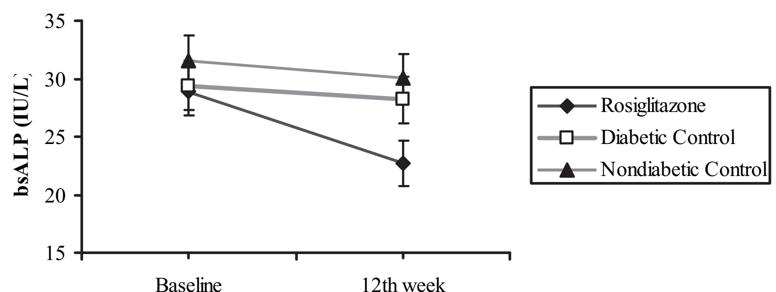

FIG. 1. Changes in serum bsALP among the rosiglitazone and control groups after 12 wk.

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TABLE 5. Baseline bivariate correlations

Baseline (n = 82)	Correlations			
	ALP	bsALP	OCL	Urine DPD
Duration after menopause	−0.074	−0.310[a]	−0.370	−0.410
Body fat (kg)	0.142	0.033	−0.222[b]	0.021
bsALP	0.634[a]		0.083	0.094
OCL	0.112			0.144
Urine DPD	0.105			
HOMA index	0.067	0.189	−0.297[a]	−0.067
HbA1c	0.179	0.022	−0.316[b]	−0.130
IL-1β	0.151	0.152	−0.205	0.176
IL-6	0.224[b]	−0.029	−0.222[b]	−0.035
TNF-α	0.007	−0.251[b]	−0.126	0.144

[a] $P < 0.01$.
[b] $P < 0.05$.

erate negative correlation with the changes in BMI and total body fat after either rosiglitazone treatment or diet in the diabetic control group. The correlations between OCL concentrations and changes in cytokines in both the rosiglitazone and diabetic control groups were negative, but not statistically significant.

Discussion

PPAR-γ activation appears to affect the differentiation of osteoblasts and/or osteoclasts because mesenchymal progenitors can also contribute to osteoblasts in addition to adipocytes.

Data on the effects of TZD administration on human bone are limited. Okazaki *et al*. (19) found that the administration of troglitazone for 4 wk decreased the biochemical markers of bone remodeling in 33 diabetic patients. However, those markers returned to their baseline values after 6 and 12 months of treatment in another study of troglitazone (15). This study was limited in that there was no control group. Data from a 4-yr follow-up of the Health, Aging and Body Composition observational study revealed that each year of TZD (troglitazone, pioglitazone, and/or rosiglitazone) use by older (age range 70–79 yr) diabetic women, but not men, was associated with a statistically significant increase in the annualized rate of whole-body bone loss (20). The rate of fracture in female diabetic subjects randomized to receive rosiglitazone was higher than that in subjects randomized to receive either metformin or glyburide, according to the results of a 4-yr study of the glycemic durability of oral monotherapies (22). However, these studies were not designed to identify the probable mechanisms by which TZDs may affect bone metabolism.

In our study, total ALP and bsALP levels, which are representative early markers of osteoblastic differentiation and bone formation during the matrix maturation phase, decreased with statistical significance after rosiglitazone administration, a result that suggests a decrease in bone turnover. These findings are consistent with those from a randomized trial that examined the effects of rosiglitazone on bone in healthy postmenopausal women (21). That study showed that after 14-wk treatment with rosiglitazone 8 mg/d, the total ALP and procollagen type I N-terminal propeptide levels, which are also markers of early bone formation that generally appear during osteoblast proliferation, decreased by 17 and 13%, respectively. These bone marker results suggest that rosiglitazone affects bone formation at an earlier stage. Recent studies in animal models have shown that rosiglitazone deflected bipotential precursor cells from osteoblast to adipocyte lineage and also attenuated the differentiation of monopotential osteoblast progenitors (13, 25, 26). This effect, which was strongly associated with the enhanced expression of PPAR-γ2, led to reduced bone formation.

There was no statistically significant change in the OCL concentration in our study. The bsALP level and OCL level also showed no statistically significant correlation. Those results may be related to the expression of these parameters

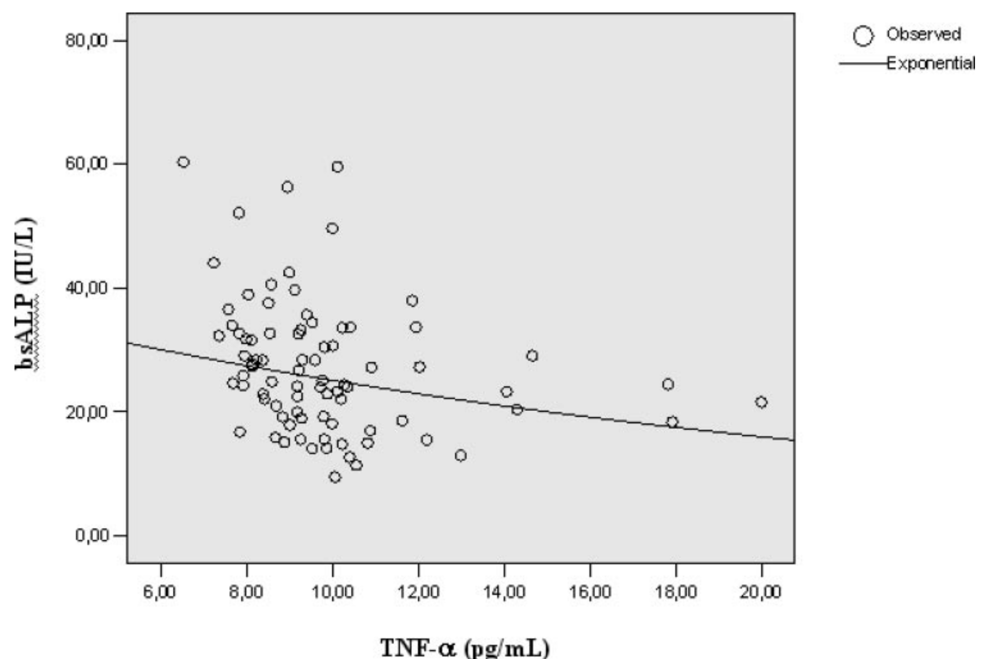

FIG. 2. Correlations between the baseline TNF-α and bsALP levels (n = 82, $r = -0.251$; $P < 0.05$).

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TABLE 6. Bivariate correlations after rosiglitazone therapy

Rosiglitazone (n = 28)	Correlations			
	ALP	bsALP	OCL	Urine DPD
BMI	0.227	0.020	−0.492[a]	0.091
Body fat (kg)	0.146	−0.045	−0.484[a]	−0.132
bsALP	0.336		0.138	−0.121
OCL	0.100			0.106
Urine DPD	0.030			
HOMA index	0.384[b]	0.316	0.034	0.315
IL-1β	0.132	−0.092	−0.293	0.04
IL-6	0.459[b]	0.115	−0.161	0.177
TNF-α	−0.157	−0.534[a]	−0.363	−0.030

[a] $P < 0.01$.
[b] $P < 0.05$.

at different stages of osteoblastic differentiation (27). OCL expressed in mature osteoblasts has been involved in the arrangement of the mineral phase in bone, both *in vitro* as a possible suppressor of mineral nucleation (28) and *in vivo* as a bone matrix mediator of osteoclast differentiation and activation (29). This theory is consistent with that advocating a probable role for the synthesis and binding of this protein to minerals in the coupling of osteoblast and osteoclast activity. Rosiglitazone has not been shown to affect osteoblastic cells at later stages of differentiation (26). The average life span of osteoblasts is 3 months, a fact that may mask a decrease in plasma OCL levels caused by the suppression of osteoblastogenesis by rosiglitazone. Grey *et al.* (21) reported a modest reduction in the OCL level after 14-wk rosiglitazone treatment, a finding that was of borderline statistical significance (p for time × treatment interaction = 0.04). Although this level of significance involves a high probability of error (5%), these data may reflect a gradual disappearance of the aforementioned masking of the decline in plasma OCL. In our study there was a negative correlation among the OCL concentration, IL-6 level, and level of HbA1c, despite the good glycemic control achieved by the patients. That finding was consistent with those of previous studies (30, 31). In addition, the levels of IL-6 and HbA1c did not change with statistical significance throughout the study, a factor that could contribute to our previously mentioned results.

In our study an analysis of DPD showed statistically significantly higher urine concentrations at 12 wk compared with baseline in the control groups. No statistically significant change was observed in the DPD level after treatment with rosiglitazone. However, the statistically nonsignificant between-group difference in DPD at 12 wk suggests that the alteration in bone metabolism caused by rosiglitazone is preceded by reduced bone formation with or without a decrease in bone resorption. It is possible that rosiglitazone uncouples osteoblast and osteoclast function during the bone remodeling process. The bone marker results reported by Grey *et al.* (21) also suggested that treatment with rosiglitazone 8 mg/d does not affect bone resorption in humans. Other available data indicate that activation of PPARγ by TZDs restrains rather than stimulates osteoclast differentiation and bone resorption (14, 15). Furthermore, 3 months of treatment with oral low-dose rosiglitazone failed to change osteoclast markers under *in vivo* conditions in a study by Soroceanu *et al.* (16). These observations could be explained in part by the type of TZD used (troglitazone *vs.* rosiglitazone) and/or the effective concentration reached at the osteoclast level.

Because locally released cytokines have played a role in the regulation of bone remodeling by inducing osteoclastic activity (6, 32) or bone formation (33), one of the main objectives of our study was to assess the association between the changes in bone turnover parameters and plasma cytokine levels after rosiglitazone therapy. Effects of TZDs are generally absent on IL-6 and variable on TNFα (34–36). Treatment with rosiglitazone 4 mg/d has exerted a potent anti-inflammatory effect at cellular and molecular levels and in the plasma of both nondiabetic obese and obese diabetic

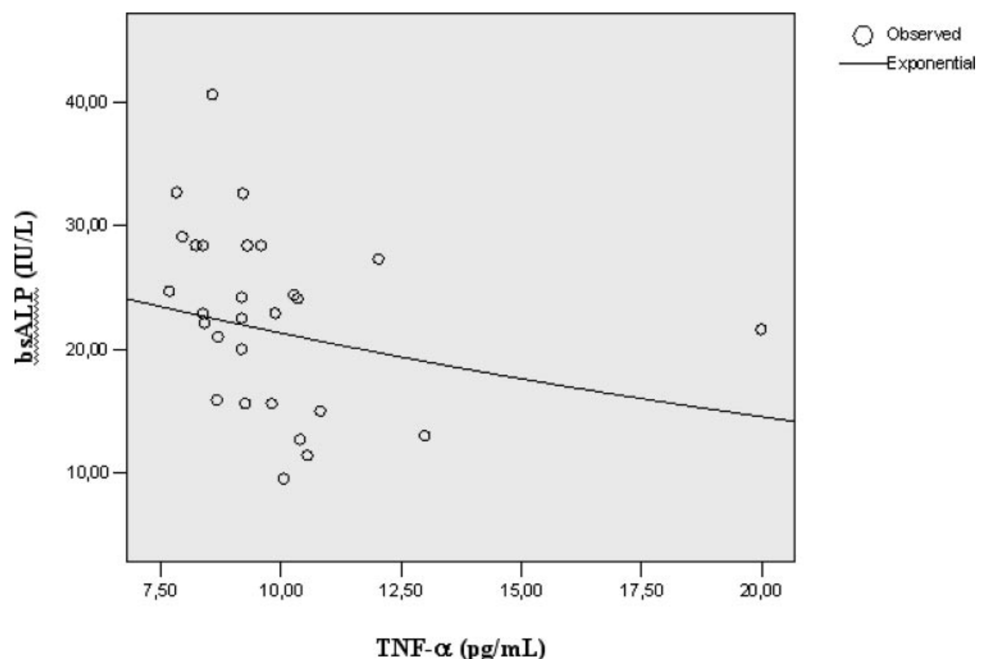

FIG. 3. Correlations between the changes in TNF-α and bsALP levels (n = 28, $r = -0.534$; $P = 0.003$) after 12-wk rosiglitazone treatment.

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subjects (37). Nonetheless, lifestyle changes have also favorably affected levels of TNF-α and IL-6 in subjects with type 2 diabetes (38, 39). Our aforementioned results pertaining to IL-1β, IL-6, and TNF-α are consistent with the findings of those studies.

Haptoglobin is also overproduced in obese individuals. IL-6 is thought to be the main cytokine that induces the synthesis of haptoglobin (40), which is synthesized predominantly in the liver. Fain *et al*. (9) demonstrated that haptoglobin is released by explants of human adipose tissue, but the contribution of extrahepatic synthesis to serum haptoglobin levels seems restricted. Adipocyte secretion of IL-6 and the subsequent hepatic synthesis of haptoglobin may explain the increase in the level of circulating haptoglobin in obese individuals (41). In our study no statistically significant changes or differences were observed in the haptoglobin level in the three groups. IL-6 levels also did not change with statistical significance, a finding that could explain the aforementioned result.

One of the important findings of our study was the observation of a moderately statistically significant negative correlation between the changes in the TNF-α level and the changes in the bsALP concentration after rosiglitazone therapy. The correlation between bsALP concentrations and changes in TNF-α was weaker and less statistically significant in the diabetic control group. That relationship has not, to our knowledge, been evaluated in other studies and has been demonstrated for the first time in our study. These observations suggest that the cytokine-lowering effects of rosiglitazone and lifestyle changes may exert some benefits on bone metabolism.

There are several limitations to our study. The study design is unconventional, and although randomized in nature, it more closely resembles an observational study. The study has a limited sample size with a considerable risk of type II error. Because physical activity and quality of life were not assessed, the analysis of the effect of rosiglitazone on bone mass could be biased. We measured serum cytokine levels, which did not reflect local bone metabolism. Some authors (42) have suggested that measurements of cytokine production by peripheral blood mononuclear cells may better reflect the capability of cells to release cytokines when stimulated. However, measurements by this way also may not reflect local effects on bone.

In conclusion, our results suggest that rosiglitazone use is associated with reduced bone formation at earlier stages in postmenopausal diabetic women, a finding reflected by the statistically significant decrease in serum bsALP concentrations after 12-wk rosiglitazone therapy. However, the negative correlation between TNF-α and bsALP levels suggests that the cytokine-lowering effect of rosiglitazone may be also involved in the remodeling process and could exert some beneficial effect on bone metabolism.

Our findings have generated a host of issues to be defined, such as: the temporal relationship of changes between different bone markers during therapy; the complex interaction between rosiglitazone, cytokines, and bone metabolism; the long-term effects of rosiglitazone therapy on bone metabolism; and whether rosiglitazone at different dosages produces different effects. Those issues require large-scale randomized placebo-controlled trials and long-term follow-up for evaluation.

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