

The Effects of Pioglitazone on Bone Formation and Resorption Markers in Type 2 Diabetes Mellitus

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Abstract

Objective The use of thiazolidinediones is reported to be associated with an increased frequency of fractures, especially in women; however, the underlying mechanism is not clear. In this prospective study, we compared the effects of pioglitazone and metformin on bone metabolism in Japanese patients with type 2 diabetes mellitus.

Methods A total of 58 patients with type 2 diabetes (24 men and 34 women) were randomly assigned to receive either pioglitazone (30 and 15 mg/day for men and women, respectively) or metformin (750 mg/day). The changes in serum and urinary type 1 cross-linked N-telopeptide (NTX), type 1 cross-linked C-telopeptide (CTX), bone alkaline phosphatase (BAP), homocysteine, and serum pentosidine were evaluated before and after three months of treatment. The primary endpoint was changes in bone resorption markers after three months.

Patients The subjects of this research were male and female type 2 diabetes patients, less than 80 years of age.

Results Pioglitazone significantly increased the serum and urinary NTX and serum and urinary CTX levels. The rates of changes in the serum and urinary NTX and CTX were significantly greater in the pioglitazone group than in the metformin group. Although the BAP levels decreased significantly in the pioglitazone group, the rates of change were similar between the two groups. In the pioglitazone group, the changes in fasting insulin levels correlated significantly with increased bone resorption, independent of age and gender.

Conclusion The results demonstrated that pioglitazone increased bone resorption independent of age and gender in Japanese patients with type 2 diabetes.

Key words: thiazolidinediones, PPAR γ , pioglitazone, type 2 diabetes, bone metabolism

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Introduction

Patients with type 1 or 2 diabetes mellitus are at a high risk of bone fractures (1). The pathological mechanism of bone metabolism in patients with diabetes mellitus is complex. Recent studies have reported that certain glucose-lowering agents affect bone metabolism (2). Among them, the most frequently reported are thiazolidinediones, which act as agonists of peroxisome proliferator-activated receptor-gamma (PPAR- γ). For example, the Health, Aging, and Body Composition Study reported a significant decrease in the bone mass in thiazolidinediones-treated older women,

but not men, with type 2 diabetes mellitus (3). In addition, a randomized controlled clinical trial (The Diabetes Outcome Progression Trial) reported a significantly higher risk (1.81- and 2.13-fold) of fracture in women, but not men, treated with rosiglitazone than in those treated with metformin and glyburide, respectively (4). While these studies revealed gender differences, a separate retrospective study found low bone mineral density in both men and women treated with rosiglitazone (5). One meta-analysis study concluded that thiazolidinediones increased the risk of fractures by 1.45- and 2.23-fold for all subjects and women, respectively (6). Another meta-analysis concluded that thiazolidinediones increased the risk of fracture in women by 1.48-fold compared

to other glucose-lowering agents (7). However, these studies were conducted in Caucasians and their findings may have limited applicability to diabetics of different ethnicities. In addition, the underlying mechanism of the increased risk of fractures was not investigated in these studies because the studies were not originally designed to evaluate the effects of glucose-lowering agents on bone in which fracture had been reported as an adverse event.

With regard to the effects of glucose-lowering agents on bone metabolism, 1 study found that treatment of non-diabetic post-menopausal women with thiazolidinedione for 14 weeks resulted in increased bone resorption and decreased bone formation, and that these changes were associated with decreased bone mineral density (8). In Japan, Kanazawa et al. (9) compared the effects of pioglitazone (a thiazolidinedione) and metformin on bone mineral density and metabolism markers. They reported that treatment with pioglitazone was associated with significantly low blood levels of osteocalcin (a bone formation marker) and low bone mineral density of the femoral neck and one-third of the distal radius (9). However, few reports have examined the effects of thiazolidinediones on bone resorption in type 2 diabetes patients. Thus, at this stage, the exact mechanism of the increased fracture risk in type 2 diabetes patients associated with thiazolidinediones remains to be elucidated.

In the present prospective study, we compared the effects of pioglitazone and metformin on bone metabolism in clinical practice using the bone metabolic markers homocysteine and pentosidine in Japanese patients with type 2 diabetes mellitus.

Materials and Methods

Study population

The subjects of this research comprised male and female type 2 diabetes patients, less than 80 years of age, who attended the First Department of Medicine at the University of Occupational and Environmental Health, Japan, and its associated centers between January 2010 and March 2012. Patients treated with pioglitazone, metformin or insulin; with type 1 diabetes or a history of secondary osteoporosis, heart failure, renal failure or liver failure; and patients receiving any drugs that affect bone metabolism (bisphosphonates, vitamin K, estrogen, calcium, anabolic steroids, or male/female hormones) were excluded from the research.

The present study was approved by the Ethics Review Committee of the University of Occupational and Environmental Health. All patients volunteered and signed an informed consent. This study was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This clinical trial was registered with the University Hospital Medical Information Network (UMIN No 000023805).

Study protocol

This was an open-labeled, prospective, randomized study. The study subjects were divided into the pioglitazone group (males 30 mg/day, females 15 mg/day) and the metformin group (750 mg/day) by the sealed envelope method. Before the start of the study and three months later, we measured the serum N-terminal telopeptide of type I collagen (NTX), urine NTX, serum C-terminal telopeptide of type I collagen (CTX), urine CTX, bone-specific alkaline phosphatase (BAP), homocysteine, pentosidine, HbA1c, fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels. During the study period, no changes in medications known to affect bone metabolism or glucose metabolism were allowed.

Efficacy and safety assessments

The primary outcomes of the study were changes in the serum and urine NTX at three months after the start of the study. The secondary outcomes were changes in the serum and urine CTX, BAP, homocysteine and pentosidine at three months after the start of the study.

Biochemical measurements

Blood and urine samples were collected after overnight fasting. The biochemical markers were measured by standard methods. Samples were stored at -30°C . We assayed stock samples at the same time. BAP was measured using an enzyme immunoassay technique (Osteolinks BAP; Quidel Corporation, San Diego, USA; fully automated EIA apparatus, Nippon Advanced Technology, Japan), serum and urine NTX were determined using an enzyme-linked immunosorbent assay (ELISA, Osteomark NTX; Alere Medical fully automated EIA apparatus, Nippon Advanced Technology), serum CTX was determined using an ELISA (FRELISA β CrossLaps-N; Fujirebio Tokyo, Japan; microplate reader spectra max puls; Molecular Devices Corporation, Tokyo, Japan) and urine CTX was determined using an ELISA (FRELISA β CrossLaps-N, Fujirebio Inc.; microplate reader spectra max puls; Labosystems Corporation, Tokyo, Japan). Homocysteine was determined using high-performance liquid chromatography (YMC-Pack Pro C18; YMC Kyoto, Japan; HPLC system, Shimadzu Corporation, Hitachi, Japan; JASCO Corporation, Tokyo, Japan), and pentosidine was determined using an ELISA kit (FSK pentosidine; Fushimi Pharmaceutical, Japan; Benchmark 1,575 Microplate Reader, Sakura Seiki Tokyo, Japan). The value for HbA1c (%) was estimated as an National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula $\text{HbA1c} (\%) = \text{HbA1c} (\text{JDS}) (\%) + 0.4\%$, considering the relational expression of HbA1c (JDS)(%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) (10). Homeostasis model-assessment of insulin resistance (HOMA-IR) was calculated as follows: $\text{FPG} (\text{mg/dL}) \times \text{FPI} (\mu\text{U/mL}) / 405$.

Table 1. Demographics and Baseline Characteristics of Diabetic Patients and Effects of Treatment with Pioglitazone or Metformin for 3 Months.

	pioglitazone			metformin		
	Baseline	3 months	% Change	Baseline	3 months	% Change
Males/females	13 / 16			11 / 18		
Age (years)	64.1±8.5			65.1±7.7		
BH (cm)	158.2±9.2			159.9±9.4		
BW (kg)	64.4±11.3	64.8±10.8	0.8±2.3	63.2±13.6	63.3±13.7	0.0±2.9
BMI (kg/m ²)	25.6±4.0			24.3±3.8		
SBP (mmHg)	122.5±12.2			123.2±12.3		
DBP (mmHg)	73.2±14.5			70.4±10.4		
HbA1c (%)	7.4±0.7	7.1±0.9**	-4.3±5.2	7.4±0.7	7.0±0.5**	-5.7±7.0
FPG (mg/dL)	130.1±26.1	111.3±18.0**	-10.3±17.7	130.8±22.8 [‡]	115.7±17.0**	-11.9±16.5
FPI (μU/mL)	7.5±4.1	6.4±3.4	-5.3±51.1	7.8±4.4	7.0±4.5	-9.9±33.3
HOMA-IR	2.4±1.3	1.8±1.1*	-8.7±70.9	2.6±1.9	2.1±1.5**	-20.3±34.3
BAP (IU/L)	21.8±7.1	20.4±7.9*	-5.8±23.3	25.7±7.8	23.7±9.4	-6.6±25.8
s-NTX (nmolBCE/L)	12.2±4.2	14.4±5.8**	17.6±23.7****	13.5±4.3	13.0±3.7	9.1±76.8
u-NTX (nmolBCE/nmol-Cre)	35.4±15.3	38.9±18.0*	11.4±27.6****	39.9±16.6	39.2±23.9	1.4±49.1
s-CTX (ng/mL)	0.5±0.3	0.5±0.2	15.8±36.6****	0.6±0.2	0.5±0.2**	-13.6±19.5
u-CTX (μg/mmpl-Cre)	185.8±116.5	210.1±111.9	26.6±50.0****	229.2±137.0	234.0±191.6	7.5±76.0
homocysteine (μmol/L)	8.4±1.8	8.8±1.9	5.0±18.3	9.9±3.1	9.7±2.8	-0.1±17.9
pentosidine (μg/mL)	0.04±0.02	0.05±0.03	15.0±43.9	0.05±0.05	0.05±0.07	7.0±32.2

Data are mean±SD or number of patients. Categorical variables were compared by χ^2 test. Although continuous variables were normally distributed.

* p<0.05 versus baseline, ** p<0.01 versus baseline by Wilcoxon matched-pairs signed-rank test. *** p<0.05 versus metformin, **** p<0.01 versus metformin by unpaired Mann-Whitney U test.

BAP: bone-specific alkaline phosphatase, BMI: body mass index, BW: body weight, DBP: diastolic blood pressure, FPG: fasting plasma glucose, FPI: fasting plasma insulin, HbA1c: glycated hemoglobin, HOMA-IR: homeostasis assessment model of insulin resistance, SBP: systolic blood pressure, s-CTX: serum c-terminal telopeptide of type I collagen, s-NTX: serum n-telopeptide of type I collagen, u-CTX: urine c-terminal telopeptide of type I collagen, u-NTX: urine n-telopeptide of type I collagen

Statistical analyses

The data are expressed as the mean ± standard deviation (SD). Between-group comparisons were tested by an unpaired Mann-Whitney U test or chi-squared test. The baseline and post-treatment values within each group were compared by Wilcoxon's signed rate test or the Friedman test. The factors influencing the percent change in serum NTX were analyzed using Spearman's rank correlation for variables. A multivariate stepwise regression analysis was conducted using the percent change in serum NTX as the dependent variable and several parameters found to be significantly related to the percent change in serum NTX on a univariate analysis. p values less than 0.05 were considered to reflect significant differences. All of the analyses were conducted using the PASW statistics analysis software program IBM SPSS v19.0 (SPSS, Chicago, USA).

Results

Patient demographics

Fifty-eight patients with type 2 diabetes met the enrollment criteria and gave their informed consent to participate in this study. Each patient was randomly assigned to a treatment arm by the sealed envelope method: 29 in the pioglitazone

group and 29 in the metformin group. All patients completed the study. The clinical characteristics of the patients are presented in Table 1. At baseline (before the start of treatment), there were no significant differences between the two groups with regard to age, male-to-female ratio, body mass index (BMI), parameters of glucose and bone metabolism.

Glucose metabolism

Table 1 shows the effects of treatment on glucose metabolism. The glycated hemoglobin (HbA1c) levels improved significantly in both the pioglitazone and metformin groups (p=0.001), and the percent changes were similar between the two groups. Both groups showed significant improvements in fasting blood glucose (p=0.011 and p=0.003, pioglitazone and metformin groups, respectively) and HOMA-IR (p=0.036 and p=0.007, pioglitazone and metformin groups, respectively) levels, and their rates of change were similar. There were no significant changes in the fasting insulin levels due to treatment.

Bone formation and resorption markers

Changes in the primary endpoint of serum and urinary NTX levels are shown in Table 1. Treatment with pioglitazone, but not metformin, resulted in a significant increase in the serum NTX level (p=0.001), with a significantly higher

Table 2. Correlation Coefficients for the Relationship between Percent Change in Serum NTX and Percent Change in Markers of Diabetic Control in Pioglitazone-treated Patients.

	r	p value
sex	-0.182	0.344
age	0.323	0.088
% change in BW	-0.513	0.015
% change in HbA1c	0.641	0.105
% change in FPG	-0.265	0.234
% change in FPI	-0.465	0.029
% change in HOMA-IR	-0.429	0.046
% change in BAP	-0.168	0.385
% change in homocysteine	0.314	0.097
% change in pentosidine	0.146	0.448

Correlation coefficients of Spearman rank correlation analysis for variables measured at 3 months after treatment.

See Table 1 for abbreviations.

BAP: bone-specific alkaline phosphatase, BW: body weight, FPG: fasting plasma glucose, FPI: fasting plasma insulin, HbA1c: glycated hemoglobin, HOMA-IR: homeostasis assessment model of insulin resistance

rate of change in the pioglitazone group than in the metformin group ($p=0.003$). In addition, pioglitazone, but not metformin, tended to increase the urinary NTX level ($p=0.053$). The rate of change in the urinary NTX level was significantly higher in the pioglitazone group ($p=0.041$) than in the metformin group (Table 1).

Table 1 show the changes in the secondary endpoints. There was no significant change in the serum CTX level in the pioglitazone group, whereas a significant decrease was noted in the metformin group ($p=0.001$). In addition, the rate of change was greater in the pioglitazone group than in the metformin group ($p=0.001$). There were no significant changes in the urinary CTX level in either group ($p=0.074$ and $p=0.216$ for the pioglitazone and metformin groups, respectively), but the rate of change was significantly greater in the pioglitazone group than in the metformin group ($p=0.021$).

Treatment with pioglitazone, but not metformin, resulted in a significant decrease in the BAP levels ($p=0.034$). The rate of changes in the BAP levels were similar between the two groups ($p=0.774$). However, there were no marked differences between men and women in the effects of pioglitazone on bone formation and resorption markers in this study.

Homocysteine and pentosidine

Treatment with metformin had no effect on the homocysteine level, while that with pioglitazone tended to reduce these levels, albeit nonsignificantly ($p=0.186$). A similar trend was noted in serum pentosidine levels. The rates of changes in both markers were similar between the two groups.

We also examined the relationship between the percent

change in the serum NTX level and the percent changes in markers of diabetic control in the pioglitazone group. A univariate analysis showed that the percent change in serum NTX correlated significantly with the percent change in body weight ($r=-0.513$; $p=0.015$), FPI ($r=-0.465$; $p=0.029$) and HOMA-IR ($r=-0.429$; $p=0.046$) (Table 2). A univariate analysis showed that the percent change in the urine NTX and serum and urine CTX levels correlated significantly with the percent change in body weight ($r=-0.438$; $p=0.042$, $r=-0.556$; $p=0.007$, $r=-0.499$; $p=0.018$, respectively), but not with FPI or HOMA-IR. Finally, a multivariate analysis identified the percent change in serum NTX as the only significant determinant of the percent change in FPI (adjusted multiple $R^2=0.217$, standardized coefficient $\beta=-0.465$, $p=0.029$) (Table 3).

Discussion

In the present study, we compared the effects of pioglitazone and metformin on bone metabolism in Japanese patients with type 2 diabetes mellitus in a prospective randomized study. While treatment with pioglitazone, but not metformin, significantly decreased BAP levels, the rates of change were similar between both treatment groups. However, only pioglitazone treatment significantly increased the levels of bone resorption markers NTX and CTX, indicating that it stimulates bone resorption in Japanese patients with type 2 diabetes mellitus. Some, but not all, previous studies have demonstrated decreases in bone formation markers with pioglitazone treatment. One report noted that osteocalcin, a bone formation marker, was decreased six months after the start of pioglitazone (9). These inconsistent results might be explained by bone resorption markers changing over weeks, whereas bone formation markers change over a period of several months. Therefore, it is possible that only bone resorption markers would have changed significantly during the three-month observation period of this study. Osteocalcin is known to correlate negatively with fasting blood glucose in patients with type 2 diabetes mellitus (11). In this study, we used only BAP as a bone formation marker and did not measure osteocalcin, which might have affected the results. Future studies should include long-term observation and the use of bone formation markers other than BAP, such as osteocalcin.

Pioglitazone acts as an insulin sensitizer and improves insulin resistance by stimulating nuclear PPAR- γ receptor. Stimulation of PPAR- γ induces differentiation of adipocytes with resultant increase in small fat cell count, apoptosis of large fat cells, hyperadiponectionemia, and hypocytokinemias of tumor necrosis factor- α (TNF- α) and reduction of free fatty acids (FFA) levels (12, 13). One study of Caucasian patients suggested that rosiglitazone (a thiazolidinedione) increased the frequency of fractures, especially in women (4). Another Japanese study also reported that postmenopausal women treated with pioglitazone were likely to experience vertebral fractures (9). A more recent meta-

Table 3. Results of Multivariate Regression Analysis with Percent Change in Serum NTX as the Dependent Variable.

Variables	Unstandardized Coefficient	Standardized coefficients β	p value	95%CI	
Intercept	-12.810		0.003	3.184	22.437
% change of FPI	-0.194	-0.465	0.029	-0.366	-0.022

Multivariate stepwise regression analysis with % change in serum NTX level as the dependent variable and sex, age, BW at baseline, % change in BW and % change in FPI as the independent variables.

BW: body weight, FPI: fasting plasma insulin, NTX: n-telopeptide of type I collagen

analysis reported that thiazolidinediones increased the risk of fracture by 1.5- to 2.5-fold (6).

However, issues of gender differences, fracture site, and the underlying mechanism of fracture remain to be clarified. Although the present study included only a small number of subjects, we observed no marked differences in markers of bone metabolism by age or gender. Thiazolidinediones activate PPAR- γ , and the over-expression of PPAR- γ in totipotent mesenchymal stem cells results in the preferential differentiation of these cells into adipocytes and the inhibition of differentiation into osteoblasts (14, 15). In contrast, an *in vitro* study reported that the activation of PPAR- γ results in the inhibition of osteoclast differentiation (16). However, a study using a mouse model (Tie2Cre/flox: osteoblast PPAR- γ [+], osteoclast PPAR- γ [-]) reported increased bone mass and reduced bone marrow space in such mice, which resulted in osteoclastic differentiation disorder and abnormal receptor activation of nuclear factor kappa-B ligand (RANKL) signal transmission. These results suggest that rosiglitazone-induced activation of PPAR- γ enhanced osteoclast differentiation and attenuated c-fos formation (17). However, there is no consensus regarding the effects of thiazolidinediones on osteoclasts, and the effects of thiazolidinediones on bone metabolism are also poorly understood.

A study in humans that examined the effects of thiazolidinediones on bone metabolism markers using samples from the Diabetes Outcome Progression Trial (ADOPT) study found no significant changes in bone formation markers. However, there was a significant increase in the levels of the bone resorption marker CTX following rosiglitazone treatment, and this increase correlated with the frequency of fractures (18). Our study also showed a significant increase in the levels of bone resorption markers. Because the positive effects on HbA1c levels were similar between metformin and pioglitazone, we hypothesize that the increased bone resorption likely represents the effects of pioglitazone. In addition, the increased bone resorption was not associated with changes in the blood glucose levels but was associated with changes in the fasting insulin levels. We consider that the improvement in hyperinsulinemia in the present study was likely due to PPAR- γ activation, although the levels of adiponectin and PPAR- γ activation were not examined. We assume that the levels of bone resorption markers reflect the effects of pioglitazone. Thus, any increase in the effect of

pioglitazone would also translate to increased bone resorption.

In the present study, while homocysteine levels tended to be increased in the pioglitazone group, pentosidine levels showed no changes. One possible explanation for this observation might be that pentosidine, an advanced glycation end products (AGE), is not a relevant bone matrix marker for our study subjects since their blood glucose was controlled. The examination of urinary pentosidine should also be considered in future studies.

The results of the present study showed that pioglitazone induces an imbalance in bone metabolism by increasing the rate of bone resorption and decreasing the rate of bone formation in Japanese patients with type 2 diabetes mellitus. We believe that clinicians should consider treatment for both diabetes mellitus and bone disease when patients with type 2 diabetes treated with pioglitazone present with abnormal levels of bone metabolism markers.

Several limitations associated with the present study warrant mention. First, it was a short-term (three months) study. Second, it included a relatively small number of patients. Such a small population necessarily limits the multiple regression and correlation analyses. Third, FPI and HOMA-IR showed significant correlations with the serum NTX levels but not with the levels of other bone resorption markers. There are a number of possible reasons for these observations. The urinary concentrations of NTX and CTX might have been higher in subjects with reduced muscle mass, since the measurements were corrected for creatinine. It is also possible that urine samples were not collected at the same time of day, which could have influenced the results. The lack of any correlation with the serum CTX, a bone resorption marker affected by food intake and the circadian rhythm, could also be due to variations in the timing of sample collection. Future studies should therefore include a strict sample collection procedure and perhaps the use of TRAP-5b, a bone resorption marker not affected by food intake, the timing of sample collection, or muscle mass. Fourth, the relationship between the changes in bone metabolism and the incidence of fractures could not be examined. Further studies are thus needed to examine this important issue.

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The authors state that they have no Conflict of Interest (COI).

References

- Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol* **166**: 495-505, 2007.
- Palermo A, D'Onofrio L, Eastell R, et al. Oral anti-diabetic drugs and fracture risk, cut to the bone: safe or dangerous? A narrative review. *Osteoporos Int* **26**: 2073-2089, 2015.
- Schwartz AV, Sellmeyer DE, Vittinghoff E, et al. Thiazolidinedione use and bone loss in older diabetic adults. *J Clin Endocrinol Metab* **91**: 3349-3354, 2006.
- Kahn SE, Kahn SE, Haffner SM, et al.; ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* **355**: 2427-2443, 2006.
- Yaturu S, Bryant B, Jain SK. Thiazolidinedione treatment decreases bone mineral density in type 2 diabetic men. *Diabetes Care* **30**: 1574-1576, 2007.
- Loke YK, Singh S, Furberg CD. Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. *CMAJ* **180**: 32-39, 2009.
- Bazelier MT, deVries F, Vestergaard P, et al. Risk of fracture with thiazolidinediones: an individual patient data meta-analysis. *Front Endocrinol (Lausanne)* **4**: 11, 2013.
- Grey A, Bolland M, Gamble G, et al. The peroxisome proliferator-activated receptor-gamma agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial. *J Clin Endocrinol Metab* **92**: 1305-1310, 2007.
- Kanazawa I, Yamaguchi T, Yano S, et al. Baseline atherosclerosis parameter could assess the risk of bone loss during pioglitazone treatment in type 2 diabetes mellitus. *Osteoporos Int* **21**: 2013-2018, 2010.
- The Committee of Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Invest* **1**: 212-228, 2010.
- Kanazawa I, Yamaguchi T, Yamamoto M, et al. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. *J Clin Endocrinol Metab* **94**: 45-49, 2009.
- Okuno A, Tamemoto H, Tobe K, et al. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J Clin Invest* **101**: 1354-1361, 1998.
- Yamauchi T, Kamon J, Waki H, et al. The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPAR gamma agonist improve insulin resistance. *J Biol Chem* **276**: 41245-41254, 2001.
- Lecka-Czernik B, Gubrij I, Moerman EJ, et al. Inhibition of *Osf2/Cbfa1* expression and terminal osteoblast differentiation by PPAR-gamma2. *J Cell Biochem* **74**: 357-371, 1999.
- Takada I, Suzawa M, Matsumoto K, Kato S. Suppression of PPAR transactivation switches cell fate of bone marrow stem cells from adipocytes into osteoblasts. *Ann N Y Acad Sci* **1116**: 182-195, 2007.
- Okazaki R, Toriumi M, Fukumoto S, et al. Thiazolidinediones inhibit osteoclast-like cell formation and bone resorption in vitro. *Endocrinology* **140**: 5060-5065, 1999.
- Wan Y, Chong LW, Evans RM. PPAR-gamma regulates osteoclastogenesis in mice. *Nature Med* **13**: 1496-1503, 2007.
- Zinman B, Haffner SM, Herman WH, et al.; ADOPT Study Group. Effect of rosiglitazone, metformin, and glyburide on bone biomarkers in patients with type 2 diabetes. *J Clin Endocrinol Metab* **95**: 134-142, 2010.

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