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Decrease in serum leptin by troglitazone is associated with preventing bone loss in type 2 diabetic patients

Sumiyo Watanabe[1], Yasuhiro Takeuchi[1], Seiji Fukumoto[2], Hiroko Fujita[3], Tadasumi Nakano[3], and Toshiro Fujita[1]

[1] Division of Endocrinology, Department of Medicine, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[2] Department of Laboratory Medicine, University of Tokyo School of Medicine, Tokyo, Japan
[3] Tokyo Metropolitan Tama Geriatric Hospital, Tokyo, Japan

Abstract The thiazolidinedione (TZD) class of antidiabetic drugs has been shown to inhibit the formation of bone-resorbing osteoclasts in vitro and to decrease bone resorption markers in vivo. These drugs also inhibit the expression of leptin in adipocytes. Less leptin can be associated with higher bone mass, based on analyses of mice deficient in leptin action. Effects of 1-year treatment with troglitazone, a member of the TZDs, on bone mineral density (BMD) and bone metabolism were examined in 25 Japanese type 2 diabetic patients. Glucose metabolism was improved, whereas body mass index and percent body fat did not change throughout the study. The percent change of BMD was negatively correlated with that of serum leptin, whereas it was not associated with changes of bone metabolic markers, type I collagen *N*-telopeptide (NTx), bone alkaline phosphatase (ALP), body mass index, or HbA1c. Serum leptin decreased in 68% of subjects (responders) after 1-month treatment and was consistently lower than the basal level throughout the treatment. Percent changes of BMD were significantly higher in the responders than in the nonresponders and in nondiabetic subjects at 6 and 12 months. NTx and bone ALP decreased at 1 month but increased thereafter in either group of patients. Thus, it is suggested that the decrease in serum leptin with no reduction in body fat mass by troglitazone is associated with preventing bone loss in type 2 diabetic patients. Hence, TZDs may have an advantage for diabetic patients who have risk factors for osteoporosis.

Key words troglitazone · thiazolidinedione · leptin · bone mineral density · diabetes mellitus

Introduction

Older women with type 2 diabetes mellitus have been reported to have an increased risk of fracture, even though their bone mineral densities (BMD) are higher than those of nondiabetic subjects [1]. Thus, fracture prevention efforts should be considered in the treatment of diabetes. A previous study indicates that improvement in glycemic control decelerates bone turnover in patients with type 2 diabetes, and that all the treatment modalities, i.e., diet therapy, oral hypoglycemic agents (sulphonyl ureas, α-glucosidase inhibitors, biguanides), and insulin, decrease indices of bone metabolism [2]. Because high bone turnover is thought to cause loss of bone mass in adults [3], and because postmenopausal women and aged men are susceptible not only to type 2 diabetes but also to osteoporosis, bone mass and bone metabolic state should be carefully monitored in type 2 diabetic patients who have risk factors for osteoporosis.

Troglitazone (Tro), a member of the thiazolidinediones (TZD), improves individual sensitivity to insulin and reduces blood glucose level in type 2 diabetes [4,5]. Apart from their antidiabetic effects, TZDs bind to and activate peroxisome proliferator-activated receptor γ (PPARγ), a member of the nuclear receptor superfamily that plays an important role in adipocytic differentiation [6]. In bone marrow, adipocytes are believed to be derived from common precursor cells that can differentiate into osteoblasts, myoblasts, chondrocytes, and fibroblasts [7–10]. Because bone marrow cells express PPARγ [11], it is plausible that TZDs such as Tro affect bone marrow cell differentiation. Indeed, it is reported that TZDs promote adipogenesis in mouse bone marrow stromal cell lines and primary mouse bone marrow cells [11–13] whereas they have few effects on osteoblastic markers [11]. TZDs also inhibits osteoclastogenesis in bone marrow cell cultures [11], although it is not known what cells in bone marrow are the immediate target of TZDs. These findings prompted us to study possible effects of TZDs on bone. In a previous study, we examined effects of Tro on metabolic bone markers in type 2 diabetes to find that treatment with Tro for 4 weeks decreased bone resorption markers [14].

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TZDs decrease the expression of leptin in adipocytes [15–17] whereas they stimulate adipogenesis [6]. Recent studies analyzing mice deficient in leptin action (e.g., *ob/ob* and *db/db*) demonstrate that less leptin can be associated with higher bone mass [18]. In humans, serum leptin level is closely correlated with body mass index (BMI) or the percentage of body fat [19], which is also associated with bone mass [20,21]; this may hamper investigating what leptin actually does for regulation of bone mass in human because leptin action deteriorates in obese subjects [19,22,23] and decrease in serum leptin level is generally associated with body fat reduction, resulting in an increase in individual sensitivity to leptin. For this reason, cross-sectional studies are not good enough to evaluate the effects of leptin on bone metabolism in humans [24–26]. However, it is still possible that a decrease in serum leptin level without body weight loss is involved in a positive effect on human bone metabolism. To address this issue, treatment with TZDs may be helpful, because Tro has been shown to suppress serum leptin level in patients with type 2 diabetes whereas it slightly increases body weight [27]. Hence, treatment with Tro may have advantages for human bone through reduction of both leptin actions and bone resorption. However, no data are available concerning in vivo effects of Tro on human bone mass. Here we present analyses in a prospective study of BMD, bone markers, and serum leptin in type 2 diabetic patients older than 60 years treated with Tro for 1 year.

Patients and methods

Patients

Twenty-five (14 female and 11 male) Japanese patients with type 2 diabetes mellitus (HbA1c 8.5% ± 0.5% and 8.3% ± 0.4%, respectively) who gave informed consent were enrolled in the present study from December 1998 to April 1999. The experimental protocol has been approved by the institutional review committee. They had not been on any drugs that are known to affect bone or calcium metabolism. Current insulin users were not admitted. Of the 25 patients, 13 had not previously been on any antidiabetic medications, 5 had been on sulphonylureas, 2 had been on α-glucosidase inhibitor, and 5 had been on both. The ages of the female patients ranged from 66 to 91 years (74.1 ± 1.9, mean ± SEM) and all were postmenopausal. The ages of the male patients ranged from 61 to 86 years (73.4 ± 2.4, mean ± SEM). All the patients took 400 mg Tro per day in two divided doses for 12 months and were instructed not to change their diet or exercise habits. One day before and 1, 6, and 12 months after starting Tro, blood and urine samples were obtained between 9 and 11 A.M. after overnight fasting. Bone mineral density (BMD) of the lumbar spine was measured before Tro administration and 6 and 12 months after starting Tro. BMD of 20 (17 female and 3 male) patients with hypercholesterolemia alone were also examined. The age of the female patients ranged from 57 to 88 years (77.1 ± 1.8, mean ± SEM) and all were postmenopausal; the age of the male patients ranged from 67 to 75 years (71.3 ± 2.3, mean ± SEM).

Measurements

BMD of lumbar spine (L2–L4) and the percentage of body fat were measured by dual-energy X-ray absorptiometry using a Hologic QDR 2000 (Waltham, MA, USA). Every operation was done by the same physician throughout the study. Fasting blood and urine samples were collected for leptin and metabolic bone markers at baseline and during Tro administration. Serum leptin was assayed by a radioimmunoassay (Human Leptin RIA; LINOCO Research, St. Charles, MO, USA). Bone-type alkaline phosphatase (ALP) was measured by an immunoassay using Alkphase-B kit (Metra Biosystems, Mountain View, CA, USA) and urinary *N*-telopeptide of type I collagen (NTx) was evaluated with an immunoassay using Osteomark NTx (Mochida Pharmaceutical, Tokyo, Japan). Assay for the metabolic bone markers and leptin were performed as paired samples, whereas the other measurements were performed on the day samples were collected.

Statistical analyses

All statistical analyses were performed using StatView software (Version 4.51.1; Abacus Concepts, Berkeley, CA, USA). The differences between the values before and after Tro treatment were analyzed by Student's paired *t* test, and those among groups were done by ANOVA. The coefficients of correlation were calculated by Pearson's method. Probability values less than 0.05 were defined as significant.

Results

Change of lumbar BMD during troglitazone treatment

Before Tro administration, *Z* score of lumbar BMD was not correlated with leptin, HbA1c, insulin, NTx, or bone ALP but with BMI (Table 1). Serum leptin level was significantly correlated with BMI as reported [19,28], whereas it was correlated with fasting serum insulin level only in male subjects. When Leptin/BMI was introduced into analyses, it gave no additional significant correlation (data not shown). Both bone markers, NTx

Table 1. Correlation matrix (calculated according to Pearson's method) between bone, glucose and their related markers

	Leptin						
	Female	Male	HbA1c	Insulin	BMI	NTx	Bone ALP
BMD (*Z* score)	0.300 (N.S.)	−0.286 (N.S.)	0.210 (N.S.)	0.079 (N.S.)	0.521 (0.0129)	−0.193 (N.S.)	0.009 (N.S.)
Leptin							
Female			−0.221 (N.S.)	−0.007 (N.S.)	0.747 (0.0034)	−0.552 (N.S.)	0.002 (N.S.)
Male			−0.136 (N.S.)	0.861 (0.0129)	0.817 (0.0022)	−0.214 (N.S.)	0.144 (N.S.)
HbA1c				−0.364 (N.S.)	−0.210 (N.S.)	0.000 (N.S.)	0.092 (N.S.)
Insulin					0.617 (0.0064)	0.119 (N.S.)	−0.055 (N.S.)
BMI						−0.079 (N.S.)	0.233 (N.S.)
NTx							0.621 (0.0009)

P values are indicated in parentheses
BMD, bone mineral density; BMI, body mass index; NTx, *N*-telopeptide; ALP, alkaline phosphatase

Fig. 1. Correlation between percent change of serum leptin and that of lumbar bone mineral density (BMD) in patients with type 2 diabetes mellitus treated with troglitazone. Percent changes of lumbar BMD after 12 months treatment with troglitazone are plotted against those of serum leptin after 1 month ($r = -0.517$, $P < 0.01$); 95% confidence limits are shown

and bone ALP, showed a significant correlation with each other (Table 1). *Z* score of lumbar BMD in each patient equivocally changed during Tro treatment, and there was no significant difference in BMD with time until 12 months (Table 2). We then analyzed the relationship between changes of BMD and serum leptin level to clarify if decrease in serum leptin had any effects on bone metabolism. Percent changes of lumbar BMD at 12 months were negatively correlated with those of serum leptin at 1 month (Fig. 1), while they were not associated with changes of other parameters related to bone and glucose metabolism including NTx, bone ALP, HbA1c, and BMI at any time points (data not shown).

Preventing bone loss along with the decrease in serum leptin in response to troglitazone

The results described here suggest that decrease in serum leptin was associated with the increase in lumbar BMD after Tro administration. Because serum leptin level is correlated with several factors, we analyzed characteristics of subjects whose serum leptin decreased and increased in response to Tro. Serum leptin decreased in 68% of subjects (71% in female and 64% in male patients) after 1-month treatment (responder group). Serum leptin level was consistently lower than baseline value in the responder group during Tro treatment (Fig. 2). In remaining patients (non-responder group), serum leptin level increased after 1 month, and equivocally changed thereafter. Baseline characteristics, leptin, HbA1c, insulin, BMI, percent body fat (%body fat), *Z* score of lumbar BMD, and bone markers were not different between the two groups (Table 2). Serum leptin levels in female subjects were higher than those in males as usual, and those were essentially

Table 2. Comparison of characteristics for type 2 diabetic patients taking troglitazone

Characteristics	Baseline	1 month	6 months	12 months	Reference value
Leptin (ng/ml), total	7.6 ± 0.9	7.3 ± 0.9	6.5 ± 0.8	6.9 ± 0.8	6.3–10.0
Female, responder	7.3 ± 1.2	6.5 ± 1.1*	6.2 ± 1.0*	6.4 ± 1.0*	
Female, nonresponder	8.1 ± 1.1	9.1 ± 1.1*	7.0 ± 1.2	8.1 ± 1.1	
Leptin (ng/mL), total	3.4 ± 0.9	3.3 ± 0.8	3.1 ± 0.7	2.8 ± 0.8	2.5–4.2
Male, responder	3.7 ± 1.3	3.1 ± 1.0*	3.0 ± 0.9*	2.3 ± 0.7*	
Male, nonresponder	3.1 ± 1.0	3.7 ± 1.1	3.6 ± 1.1	3.3 ± 0.8	
HbA1c (%), total	8.4 ± 0.3	7.8 ± 0.3*	6.9 ± 0.2*	7.5 ± 0.3*	4.3–5.8
Responder	8.3 ± 0.4	7.6 ± 0.2*	6.8 ± 0.2*	7.3 ± 0.2*	
Nonresponder	8.5 ± 0.5	8.1 ± 0.6	7.2 ± 0.6*	8.0 ± 0.7	
Insulin (μU/mL), total	4.8 ± 0.7	N.D.	N.D.	N.D.	<15
Responder	4.6 ± 0.9	N.D.	N.D.	N.D.	
Nonresponder	5.2 ± 1.3	N.D.	N.D.	N.D.	
BMI, total	23.6 ± 0.7	N.D.	23.8 ± 0.7	23.9 ± 0.7	
Responder	23.6 ± 0.8	N.D.	23.8 ± 0.9	23.8 ± 0.9	
Nonresponder	23.6 ± 1.2	N.D.	24.0 ± 1.5	24.0 ± 1.4	
Percent body fat, total	31.5 ± 2.2	N.D.	30.9 ± 2.2	31.0 ± 2.2	
Responder	31.5 ± 2.9	N.D.	30.6 ± 2.9	30.6 ± 3.0	
Nonresponder	31.6 ± 3.3	N.D.	31.5 ± 3.0	31.6 ± 3.1	
BMD (*Z* score), total	0.83 ± 0.26	N.D.	0.87 ± 0.26	0.83 ± 0.26	
Responder	0.85 ± 0.33	N.D.	0.93 ± 0.34	0.89 ± 0.34	
Nonresponder	0.80 ± 0.43	N.D.	0.73 ± 0.42	0.71 ± 0.42	
NTx (nmol BCE/mmol creatinine), total	38.9 ± 3.9	33.3 ± 3.2*	41.3 ± 3.9	40.8 ± 3.3	<55
Responder	39.8 ± 5.2	33.9 ± 4.5	42.0 ± 5.1	41.1 ± 4.5	
Nonresponder	36.3 ± 2.7	32.1 ± 2.1	35.6 ± 4.1	39.8 ± 3.4	
Bone ALP (U/l), total	24.8 ± 1.3	22.6 ± 1.2*	23.0 ± 1.0*	24.7 ± 1.5	10.0–27.0
Responder	24.6 ± 1.5	22.3 ± 1.5*	22.7 ± 1.3	23.4 ± 1.8	
Nonresponder	25.2 ± 1.7	23.6 ± 1.5	23.7 ± 1.4	27.4 ± 2.6	

Patients whose serum leptin level decreased and increased at 1 month of treatment are in the responder and nonresponder groups, respectively; data are means ± SE
* Significantly different from the baseline values ($P < 0.05$)
BCE, bone collagen equivalent

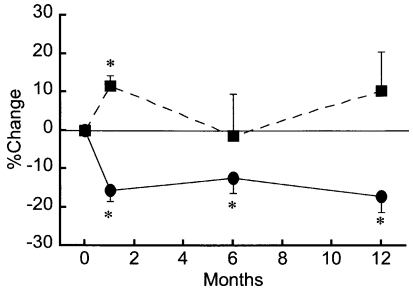

Fig. 2. Effect of troglitazone on serum leptin level in patients with type 2 diabetes mellitus. Subjects were divided into two groups, those whose serum leptin decreased (responder group, *circles*) and increased (nonresponder group, *squares*) after 1-month treatment with troglitazone (Tro). Percent changes of serum leptin from the baseline values before Tro administration are plotted. *Significantly different from the baseline ($P < 0.05$)

within a reference range before Tro administration. HbA1c was significantly lower than baseline level until 12 months in the responder group as well as in total subjects. Although HbA1c apparently decreased in the non-responder group, the reduction reached statistical significance only at 6 months. BMI and %body fat did not change until 12 months in either group. Both urinary excretion of NTx and serum bone ALP decreased at 1 month but increased thereafter in patients whose serum leptin level either increased or decreased (Table 2). Serum calcium and phosphorus level did not change during treatment with Tro (data not shown).

Z score of lumbar BMD was not significantly altered until 12 months, although there was a trend of increase and decrease in BMD in the responder and nonresponder groups, respectively. Changes of BMD in the responder group were significantly higher than those in the nonresponder group and those in the nondiabetic hypercholesterolemic group without Tro administration at both 6 and 12 months (Fig. 3). Percent changes of BMD in the latter two groups were not significantly different.

Discussion

This is the first prospective report concerning the in vivo effects of Tro on bone and indicates that Tro treatment is associated with conserving bone mass in type 2 diabetic patients whose serum leptin level decreased in

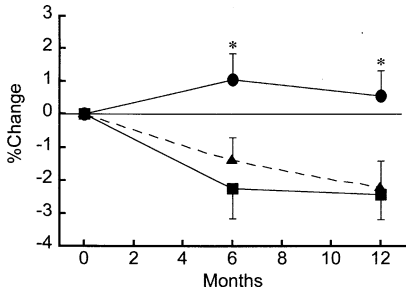

Fig. 3. Effect of troglitazone on lumbar BMD in patients with type 2 diabetes mellitus. Subjects were divided into two groups, those whose serum leptin decreased (responder group, *circles*) and increased (nonresponder group, *squares*) after 1-month treatment with troglitazone as shown in Fig. 2. Percent changes of lumbar BMD from the baseline values before Tro administration are plotted; those in nondiabetic patients with hypercholesterolemia with a similar range of ages are also plotted (*triangles*). *Significantly higher than other two groups at 6 and 12 months ($P < 0.05$)

response to Tro administration. TZDs including Tro inhibit osteoclastogenesis through their direct effects on bone marrow cells [11], and Tro administration for 4 weeks indeed decreases bone resorption markers in type 2 diabetic patients [14]. Consistent with these observations, the bone metabolic markers, urinary NTx and serum bone ALP, decreased with Tro administration at 1 month in this study. However, both markers increased thereafter, and their changes at any time point did not correlate with that of lumbar BMD at 6 and 12 months. This result suggests that suppression of bone resorption by Tro is transient in vivo and is not a primary cause of the change of BMD, although it may contribute to prevent bone loss to some extent.

Improvement of glucose metabolism was generally observed in both groups of patients whose serum leptin level increased or decreased in response to Tro administration and was not correlated with the change of lumbar BMD. However, the decrease of HbA1c was more prominent in the responder group than in the nonresponders. Thus, the improvement of glucose metabolism with Tro might be involved in its effects on bone, and the decrease in serum leptin level in the responder group might indicate that efficacy of Tro was greater than that in the nonresponders. Then, the effect of Tro might result in a positive balance of bone metabolism through the improvement of glucose metabolism or insulin action [4,29]. This hypothesis is, however, unlikely, because changes of lumbar BMD in the nondiabetic hypercholesterolemic group without Tro administration were significantly lower than those in the responders whose serum leptin decreased during the treatment. In addition, changes of lumbar BMD in the nondiabetic group were similar to those in the nonresponder group. This result suggests that changes of bone mass in type 2 diabetic patients is similar to those without impaired glucose metabolism if there is no decrease in serum leptin level. Thus, it is rather plausible to assume that the decrease in serum leptin by Tro administration is primarily involved in preventing bone loss.

Leptin is a secretory protein synthesized by adipocytes that controls body weight by inhibition of eating behavior through its effects on the central nervous system [23,30]. Furthermore, recent studies have demonstrated that leptin suppresses bone formation via its effect on the central nervous system in mice [18], although the exact mechanism of action of leptin is unclear. In human, serum leptin level is not generally correlated with such action, because leptin action has deteriorated in obese subjects and decrease in serum leptin level is usually associated with body fat reduction that may cause an increase of individual sensitivity to leptin [19,22]. Probably for this reason, there are no convincing data concerning a relationship between serum leptin level and bone mass [24–26,31], both of which are tightly associated with BMI.

Our results also demonstrated no significant correlation between serum leptin level and lumbar BMD at baseline evaluations in type 2 diabetic patients whereas either parameter correlates with BMI. Thus, it is hard to imagine that leptin is a major determinant for bone mass in humans. However, we cannot exclude the possibility that a decrease in serum leptin level without body weight loss is involved in a positive effect on human bone metabolism. TZDs decrease the expression of leptin in adipocytes [15–17], whereas they stimulate adipogenesis [6]. Tro has been reported to suppress serum leptin level in patients with type 2 diabetes along with a slight increase in body weight [27]. Actually, Tro decreased serum leptin level with no loss of adipose tissue in a majority of subjects in this study. This effect of Tro may allow us to evaluate effects of circulating leptin on human bone in patients treated with Tro, although there are many confounding factors. Results shown here demonstrate that decrease of serum leptin by Tro was associated with preventing bone loss in type 2 diabetic patients. It is, thus, suggested that leptin negatively affects bone mass in humans as in mice [18]. Taken together, although serum leptin level may not be a major determinant for bone mass, the decrease of leptin action may cause not only gain of body weight but also conservation of bone mass [32].

We cannot explain why some patients responded to Tro by a decrease in their serum leptin level but others did not. Improvement of glucose metabolism was slightly better in the responder group, suggesting actions of Tro being better in this group. This notion is consistent with our assumption that the decrease of serum leptin level is theoretically expected in patients taking Tro, because TZDs including Tro inhibit the

expression of leptin in adipocytes [15–17]. Then, the TZD class of drugs may be helpful to conserve bone mass, and this anticipation is more convincing if serum leptin decreases in response to taking such a drug.

In conclusion, it is suggested that the decrease in serum leptin with no reduction in body fat mass by Tro in addition to the transient decrease in bone metabolism is involved in preventing bone loss in type 2 diabetic patients. Hence, TZDs may have an advantage for diabetic patients who have risk factors for osteoporosis.

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