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ORIGINAL ARTICLE

Elevated vitamin D status in postmenopausal women on thiazolidinediones for type 2 diabetes

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Abstract Thiazolidenediones (TZD) have been reported to lead to non-vertebral bone loss in postmenopausal women with diabetes, but the true incidence of vertebral fractures has been under-detected because two-thirds of vertebral fractures are silent. TZD is also related to increased adiposity, with a consequently greater risk of vitamin D deficiency—both of which seem to aggravate the untoward effect of TZD on bone. The aim of this study is to determine whether TZD use is associated with prevalence of vertebral fractures and low vitamin D status in postmenopausal women with type 2 diabetes. A group of 102 postmenopausal women with type 2 diabetes, 52 TZD users for at least 12 months, and 50 non-TZD users were enrolled in the study. Any data regarding diabetes, age at menopause, co-morbidities, and drug use were recorded. Blood sampling and thoraco-lumbar radiography were performed. Bone mineral density (BMD) of L2–L4 and the femur were measured by dual-energy X-ray absorptiometry (DXA). The occurrence of vertebral fractures at one level or more in subjects on TZD was higher than those not on TZD, but did not reach statistical significance (19.2 vs. 14.0%, $P = 0.5$). Total hip BMD in subjects on TZD was significantly lower than those not on TZD (0.96 ± 0.15 vs. 1.02 ± 0.11; $P < 0.05$). Levels of 25(OH)D in TZD users were significantly higher (35.3 ± 1.5 vs. 25.9 ± 1.2 ng/dl; $P < 0.001$). The prevalence of vitamin D deficiency was 75.5% in subjects not on TZD compared to 34.6% in those on TZD (OR 6.4, 95% CI 2.6–15.6). Higher circulating 25(OH)D was observed in TZD users. TZD use was associated with lower total hip BMD but not with vertebral fracture.

Keywords Thiazolidenediones · Vertebral fractures · Bone mineral density · Osteoporosis · Postmenopausal · Morphometry · Vitamin D

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Introduction

Thiazolidinediones (TZD) are widely used in the treatment of type 2 diabetes. TZD use is associated with accelerated bone loss (0.6–1.2% per year) at the trochanter, whole body, and lumbar spine in diabetic women [1]. Concerns have recently been raised about a small increase in the risk of fractures associated with TZD at peripheral skeletal sites [2]. It is of note that increases in fractures at common sites for fragility fractures, such as the spine and hip, were not reported. Vertebral fractures are frequently undetected since only 30–40% are symptomatic and come to clinical attention. Nevertheless, asymptomatic vertebral fractures are also associated with subsequent adverse health outcomes [3]. A recent study performed on Caucasian males focused specifically on the risk of vertebral fractures associated with TZD, and found a significant increase in vertebral fractures in subjects on TZD [4]. It remains unclear whether and how TZD might affect the risk of vertebral fractures in females of other ethnicity.

Calcium and vitamin D play important roles in the maintenance of bone health. At present, vitamin D deficiency is highly prevalent [5]. Obesity is a recognized risk factor of vitamin D deficiency, which may be related to the sequestration of vitamin D in adipose tissue [6]. The relationship between the use of TZD and vitamin D status is currently unknown. We hypothesized that TZD, by increasing adiposity, may adversely affect vitamin D status and lead to further aggravation of the untoward effect of TZD on bone. To that end, we investigated, in a case–control study, the difference in the prevalence of vertebral fractures and vitamin D status between postmenopausal women on TZD and those not on TZD.

Materials and methods

Subjects

We enrolled by consecutive medical record review 102 postmenopausal women, as defined by cessation of menstruation for at least 12 months, with type 2 diabetes at the outpatient clinic of Ramathibodi Hospital, Thailand, between January 2009 and December 2009. Of the 102 subjects, 52 had been on TZD for at least 12 months; the other 50 subjects, matched for age and body mass index (BMI), had never been on TZD. All were ambulatory and non-alcoholic. Subjects were excluded in cases of: a history of cancer, hyperthyroidism, hyperparathyroidism, surgical menopause, use of oral or parenteral glucocorticoid ($\geq$5 mg prednisolone or equivalent/day) for more than 1 month within 6 months before study entry, or an estimated glomerular filtration rate <60 ml/min. No subjects had been receiving medications known to interfere with bone metabolism—including estrogen, vitamin D, antiresorptive agents, or thyroxine—within the past 12 months. The study protocol was approved by the local Institutional Review Board. All patients gave signed informed consent before participating in the study.

BMD measurements

Body weight was measured with subjects wearing light clothes. Bone mineral density (BMD) at the lumbar spine 2–4 (L2–L4) and the femur were measured by dual-energy X-ray absorptiometry (DXA) (Lunar Corp., USA) by a single experienced technician. Quality control was achieved by daily calibration and phantom scans. The coefficient of variation for the phantom scans was 0.6%; these values were 1.2 and 1.6% at L2–L4 and the femoral neck, respectively.

Ascertainment of vertebral fractures

Ascertainment of vertebral fractures was performed according to a previously described method [7]. Lateral and anteroposterior thoraco-lumbar radiographs centered at the T12 level were performed on all subjects. Vertebral fractures were evaluated by a single radiologist who was blind to the subjects' clinical characteristics. Anterior and posterior heights of vertebral levels T10–L3 were measured, and the anterior/posterior height ratio was calculated. Vertebral fracture was defined as present if the height ratio was 0.8 or lower.

25(OH)D measurement

Serum 25(OH)D levels were measured by high performance liquid chromatography, with an intra-assay precision of 4.8% for 25(OH)D2 and 4.9% for 25(OH)D3.

Statistical analysis

The sample size was determined based on a power of 0.8 to detect a 2-fold increase in vertebral fracture risk from a baseline prevalence of 15%. Pearson's chi-square or Fisher's exact tests were used to compare characteristics between groups for categorical data. Student's t test was used for continuous variables to test for any statistical differences between groups. Linear relationships between BMI and vitamin D status were assessed by linear regression analysis. Independent risk factors for vitamin D insufficiency were determined by stepwise logistic regression. Statistical significance was set at $P < 0.05$. Data were presented as mean $\pm$ SE unless stated otherwise.

Results

Baseline characteristics of the subjects are shown in Table 1. There were no significant differences in age, menopausal age, and body mass index (BMI) between the two groups. With regard to biochemical and hormonal tests, there were no significant differences in baseline HbA1c level, serum TSH, calcium, LDL cholesterol, and eGFR between the two groups. The duration of TZD use was 42.3 $\pm$ 3.2 months. The mean doses were 23.8 $\pm$ 1.2 mg ($n = 41$) and 4.4 $\pm$ 0.4 mg ($n = 11$) for pioglitazone and rosiglitazone, respectively. Subjects using TZD had a longer duration of diabetes than those not using TZD. Pioglitazone was prescribed in 41 (78.8%) subjects, while rosiglitazone was used in the remaining 11 (21.2%) subjects. The mean duration of TZD use was 3.47 $\pm$ 1.89 years (median 3.22 years). Sulfonylurea was more

Table 1 Characteristics of study subjects stratified by TZD use

	TZD users ($n = 52$)	Non-TZD users ($n = 50$)	P value
Age (year)	59.3 ± 0.9	58.4 ± 0.9	NS
BMI (kg/m^2)	29.1 ± 0.7	29.2 ± 0.6	NS
Duration of diabetes (year) median (min, max)	11.3 (2.0, 29.0)	6.8 (1.3, 26.6)	<0.01
Age of menopause (year)	50.3 ± 0.5	50.2 ± 0.5	NS
A1C (%); mean(SE)	7.8 ± 0.1	7.5 ± 0.2	NS
GFR (ml/min/1.73 m^2); mean(SE)	89.2 ± 3.4	84.9 ± 2.7	NS
Serum calcium (mg/dl); mean(SE)	9.4 ± 0.1	9.4 ± 0.0	NS
Serum TSH (μIU/ml); median(min, max)	1.5 (0.4, 4.8)	1.5 (0.4, 5.7)	NS
Serum LDL (mg/dl)	104.0 ± 4.6	111.8 ± 4.0	NS
Serum total 25(OH)D level (ng/ml)	35.3 ± 1.5	25.9 ± 1.2	0.000
Subjects using other antidiabetic medications			
Insulin	12 (23.1%)	12 (24.0%)	NS
Metformin	49 (94.2%)	45 (90.0%)	NS
Sulfonylurea	43 (82.7%)	30 (60.0%)	<0.01
Alpha-glucosidase inhibitor	5 (9.6%)	5 (10.0%)	NS

NS not significant

widely used than other antidiabetic agents by patients treated with TZD (82.69 vs. 60.0%; $P = 0.01$).

Table 2 shows BMD at the lumbar spine (L2–L4), femoral neck, and total hip, and the prevalence of vertebral fractures. There was no difference in BMD of L2–L4 and the femoral neck between the two groups. However, total hip BMD in subjects on TZD was significantly lower than in those not on TZD ($P < 0.05$). The prevalence of vertebral fractures at one level or more in subjects on TZD tended to be higher, but did not reach statistical significance (19.2 vs. 14.0%, $P = 0.5$).

The 25(OH)D levels in TZD users were significantly higher (35.3 ± 1.5 vs. 25.9 ± 1.2 ng/dl, $P < 0.001$), as shown in Fig. 1. The prevalence of vitamin D insufficiency, as defined by 25(OH)D less than 30 ng/dl, was 34.6% in subjects on TZD compared to 75.5% in those not on TZD ($n = 49$). In a stepwise logistic regression model, including TZD, BMI, age, and HbA1c as independent variables, it was found that use of TZD and BMI were both independent risk factors for vitamin D insufficiency (Table 3). To further explore the influence of TZD on vitamin D status, we evaluated the relationship of vitamin D to BMI according to TZD usage. There was a negative association between 25(OH)D levels and BMI, but the relationship did not reach statistical significance. At each level of BMI, however, 25(OH)D was approximately 10 ng/dl higher in the presence of TZD (Fig. 2).

Discussion

It is currently recognized that TZD is associated with an increase, albeit small, in the risk of fractures. Fractures associated with TZD are mostly peripheral, such as hand and foot fractures. The underlying basis for the increase in fractures at the periphery rather than at more typical sites for osteoporotic fractures is not entirely clear. However, more common types of osteoporotic fractures, particularly vertebral fractures, could be underreported since such fractures are mostly asymptomatic.

In this study, we demonstrated that asymptomatic vertebral fractures were not more common in patients on TZD as compared to those on other anti-diabetic agents. This is in contrast to a previous study showing increased vertebral

Table 2 BMD and vertebral fractures in TZD and non-TZD users

	TZD users ($n = 52$)	Non-TZD users ($n = 50$)	P value
L2–L4 BMD (g/cm^2)	1.14 ± 0.19	1.18 ± 0.20	NS
Femoral neck BMD (g/cm^2)	0.88 ± 0.14	0.92 ± 0.14	NS
Total hip BMD (g/cm^2)	0.96 ± 0.15	1.02 ± 0.11	<0.05
Subjects with vertebral fractures at one level or more	10 (19.2%)	7 (14.0%)	NS
Subjects with vertebral fractures at two levels or more	2 (3.8%)	3 (6.0%)	NS

NS not significant

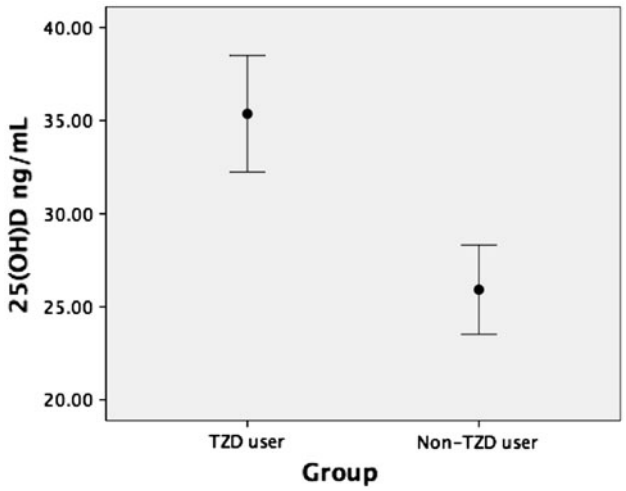

Fig. 1 25(OH)D levels in TZD versus non-TZD users (mean ± SE). *TZD* thiazolidinediones

Table 3 Characteristics associated with vitamin D insufficiency

	Odds ratio (95% CI)
Non-TZD user	6.4 (2.6–15.6)
BMI (kg/m^2)	1.110 (0.998–1.234)
Age (years)	NS
HbA1c (%)	NS

CI confidence interval, *NS* not significant

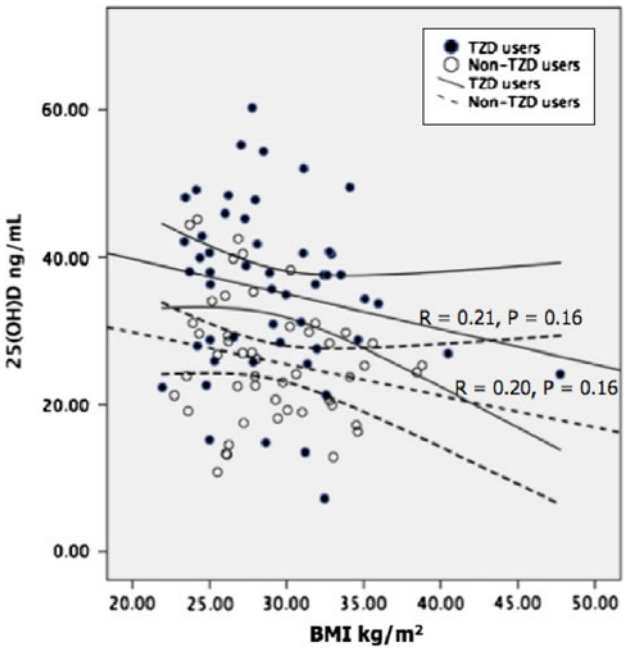

Fig. 2 Relationship between 25(OH)D levels and BMI in TZD and non-TZD users by linear regression

fractures in male TZD users [4]. A number of differences in study design may be accountable for the contradictory results. Our study group consisted of postmenopausal females, whereas only males were included in the other study. It has been demonstrated that TZD can affect the bone of both sexes differently, in that females tend to have a greater risk of fractures associated with TZD [8]. This may be due to the influence of estrogen deficiency which makes postmenopausal more susceptible to osteoporosis and osteoporotic fractures in general. It therefore seems counterintuitive that an increased propensity for vertebral fractures was not observed in this study. However, our study population was relatively young, which may render the increased risk, if any, less apparent [9].

Pioglitazone was more frequently used in our study. Both pioglitazone and rosiglitazone act through peroxisome proliferator-activated receptor gamma (PPARG) activation, but may possess differential biological functions due to the difference in the activation of other receptors. Although it has been suggested that pioglitazone causes a greater incidence of adverse skeletal effects than rosiglitazone [10], this notion is not without dispute [11]. Therefore, the lack of effect on vertebral fractures in this study is not likely to be attributed to the more common use of pioglitazone versus rosiglitazone.

There were a number of limitations in this study. Our sample size may not have enough statistical power to significantly demonstrate a small increase in vertebral fracture risk. Although subjects were well matched for age and body weight, medications for diabetes were not controlled and there was an imbalance in diabetic medications used between the two groups. In particular, sulfonylureas were used more often in the TZD group. However, it is less likely that this would affect our results since sulfonylureas, despite its long history of use in the management of diabetes, have not been associated with increased fracture risk. Lastly, TZD users had longer duration of diabetes which may more adversely affect bone metabolism.

In this study, we found that 25(OH)D levels in patients with type 2 diabetes were higher in TZD users. It is well recognized that fat mass affects vitamin D status, and low 25(OH)D levels are more commonly found in subjects with higher adiposity [12–14] as well as in those with metabolic syndrome [15]. This is likely due to sequestration of most vitamin D, a fat-soluble vitamin, in adipose tissue [16], although alteration in the metabolism of vitamin D in adipose tissue cannot be entirely ruled out. The difference in fat mass is less likely to be the cause of higher 25(OH)D associated with TZD use in this study since both the groups of study subjects were well matched for BMI. Moreover, the direction of association was opposite to what would be expected, in that TZD users had higher rather than lower vitamin D status. Although a difference in sun exposure

could account for the difference in vitamin D status, the degree of difference is not likely to be enough to be attributable to the higher vitamin D status in the TZD group. Both the groups of subjects were active, ambulatory, and without other major complications which could lead to limited sun exposure. Moreover, sun exposure behavior as assessed by questionnaires, although interpretable in certain studies [17], only explain a minor part of the variation in vitamin D status together with a number of clinical risk factors [18]. It is also likely that TZD may affect dermal vitamin D metabolism. Vitamin D synthesis takes place in the dermal/epidermal junction. It is of note that besides being present in the liver, dermal fibroblasts also express 25-hydroxylase enzymes [19] and produce 25(OH)D upon UVB exposure [20]. PPARG is ubiquitous, with myriad biological effects. It is present in dermal fibroblasts and affects a number of their biological functions [21–23]. We therefore hypothesize that the increase in 25-hydroxylation of vitamin D synthesized from the skin in TZD users may be the underlying basis of the higher vitamin D status observed with TZD use. Further studies to explore the issue are warranted and could lead to increased understanding of the metabolism of vitamin D in the skin.

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