Curcumin protects bone biomechanical properties and microarchitecture in type 2 diabetic rats with osteoporosis via the TGFβ/Smad2/3 pathway

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Received September 18, 2019; Accepted April 24, 2020

DOI: 10.3892/etm.2020.8943

Abstract. Type 2 diabetic osteoporosis (T2DOP) has become a common secondary cause of osteoporosis that accelerates bone loss and leads to bone fractures. The aim of the current study was to investigate the association between the anti-osteoporotic effect of curcumin (Cur) and the transforming growth factor (TGF)β/Smads signaling pathway. Male Sprague-Dawley rats were used in the experiments. The type 2 diabetes mellitus (T2DM) animals were treated with Cur for 8 weeks and blood lipid markers, bone microstructure and bone biomechanics were then evaluated. The mRNA expression levels of TGFβ1, type I TGFβ receptor (TβRI), TβRII and Smad2/3 were determined using reverse transcription-quantitative PCR (RT-qPCR) and immunohistochemistry. The body weight of rats with type 2 diabetes-induced osteoporosis increased (P<0.05), while the lipid (total cholesterol, triglyceride and low-density lipoprotein) and fasting blood glucose levels were decreased by Cur (P<0.05). In addition, Cur significantly improved bone biomechanical properties (maximum load, breaking load, elastic load and the bone rigidity coefficient) and preserved bone microarchitecture (P<0.05). The RT-qPCR and IHC results revealed that Cur increased TGFβ1, TβRI, TβRII and Smad2/3 expression levels and promoted Smad2/3 phosphorylation in bones. The present results also indicated that Cur regulated lipid and glucose levels, improved bone biomechanical properties and preserved bone microarchitecture, and that these effects may be mediated via TGFβ/Smad2/3 pathway activation.

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Key words: curcumin, type 2 diabetes, osteoporosis, bone biomechanics, bone microarchitecture

Introduction

Type 2 diabetic osteoporosis (T2DOP) is a severe chronic complication that affects the skeletal system and is caused by diabetes (1). T2DOP has become a common secondary cause of osteoporosis that accelerates bone loss and leads to bone fractures (1). The incidence of T2DOP fracture has been reported to be as high as 50% worldwide in 2015 and continues to rise with the increasing age of patients with islet function failure, resulting in major socioeconomic burden and a serious decline in quality of life (2). Chronically poor control of blood glucose and lipid levels are important factors that lead to osteoporosis in patients with type 2 diabetes mellitus (T2DM) (3). Long-term hyperglycemia affects bone formation and bone resorption, leading to osteoporosis (3). Furthermore, high triglyceride (TG) and total cholesterol (TC) levels reduce bone density in patients with diabetes (3). Active control of blood glucose and blood lipid is conducive to early prevention of osteoporosis (3).

There are currently no specific drugs for the clinical treatment of T2DOP (4). However, basic treatment principles are comprised of primary disease control, calcium supplements and vitamin D (5). Currently, osteoporosis treatments, including bisphosphonates and calcium supplements, are not

considered as the first choice of treatment for secondary osteoporosis and may have severe side effects (6).

Extensive research has revealed that certain Chinese herbs may help preserve bone structure and regulate lipid levels (7,8). Curcumin (Cur) is a natural polyphenolic compound extracted from the roots of the genus *Curcuma* in the dry ginger family (9). Cur has been reported to exhibit bone-protective properties in post-menopausal osteoporosis (10,11) and to prevent bone loss by inhibiting osteoclasts in a diabetic and osteoporotic animal model (12). However, the effect of Cur on T2DOP remains unclear. Furthermore, the effect of Cur on bone microstructure requires further investigation. A previous study demonstrated that Cur can inhibit Smad2/3 phosphorylation caused by transforming growth factor (TGF) β1 signaling by upregulating Smad7 in hepatic stellate cells, thus exerting an anti-liver fibrosis effect (13). However, whether the anti-osteoporotic effect of Cur is associated with the TGFβ/Smad signaling regulation pathway has not been previously reported.

Therefore, the aim of the current study was to comprehensively investigate the effect of Cur on osteoporosis in T2DM rats by observing the 3D structure of bone microstructure and by evaluating bone microstructure, bone biomechanics, serum bone conversion metabolism, blood glucose and blood lipid indicators. Furthermore, the effect of Cur on TGFβ1, type I TGFβ receptor (TβRI), TβRII and Smad2/3 expression in T2DOP rats was observed, and the association between expression changes and their anti-osteoporotic effects was assessed.

Materials and methods

Drugs and reagents. Cur was purchased from Vientiane Tianjin HengYuan Technology Co., Ltd., calcitriol (Cal) from Roche Diagnostics (Shanghai) Co., Ltd. and streptozotocin (STZ) from Sigma-Aldrich (Merck KGaA). High-sugar and high-fat fodder (57.3% carbohydrate, 20% fructose, 10% lard, 2.5% cholesterol, 10% egg yolk and 0.2% sodium cholate) was purchased from the Animal Experimental Center of Southern Medical University (Guangzhou, China).

TC (cat. no. A111-1-1), TG (cat. no. F001-1-1) and low-density lipoprotein cholesterol (LDL-C; cat. no. A113-2-1) assay kits and serum osteocalcin (OCN; cat. no. H152) and C-terminal type-I peptide (CTX-I; cat. no. H287) ELISA kits were purchased from Nanjing Jiancheng Bioengineering Institute. Smad2/3 (cat. no. TA347074; 1:1,000) and phosphorylated (p)-Smad2/3 (cat. no. TA501728; dilution, 1,000) antibodies were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.

Experimental animals. The animal experiments were approved by the Ethics Committee of Zhaoqing Medical College. A total of 50 male Sprague-Dawley rats (age, 8 weeks; weight; 180±20 g) were obtained from the Southern Medical Experimental Animal Center (certification no. SCXK 2017-0012). The animals were allowed to acclimatize to the laboratory conditions for 7 days before experiments and were housed at 25±2˚C and a relative humidity of 60-70% with 12 h light/dark cycles. The animals had access to dry pellet food and water *ad libitum*.

Induction of diabetes with high-sugar, high-fat diet and STZ. A total of ten rats were assigned to the control group and were administered a high-sugar, high-fat diet only for 12 weeks. A total of 40 rats were used to establish the T2DM model. According to previous literature (14), these rats were administered a high-sugar, high-fat diet for 4 weeks. After 4 weeks, each rat received an intraperitoneal injection of 3% STZ (40 mg/kg) (15). Rats with fasting blood glucose (FBG) ≥7.0 mmol/l were selected for further study.

Experimental design. The rats continued to receive the high-sugar, high-fat diet throughout the course of the present study. The animals were divided into five groups (n=10/group) and received the following treatments for 8 weeks (Table I): Control, T2DM, T2DM treated with 110 mg/kg/day Cur (T2DM + Cur), T2DM treated with 0.045 μg/kg/day Cal (T2DM + Cal) and T2DM treated with 200 mg/kg/day metformin (T2DM + Met; cat. no. 317240; Sigma-Aldrich; Merck KGaA). As Cur and Cal are insoluble in water (16,17), their suspension was prepared with 1% sodium carboxymethyl cellulose(cat. no. 419273; Sigma-Aldrich; Merck KGaA). The control and T2DM groups were given 1% sodium carboxymethyl cellulose (5 ml/kg) daily by oral gavage and the T2DM + Cur, T2DM + Cal and T2DM + Met groups were given their respective drugs (5 ml/kg) by oral gavage. FBG and body weight were measured once weekly for 8 weeks.

Sample collection and application. At the end of the experiment, all rats were euthanized via cardiac puncture under anesthesia with intraperitoneal injection of 45 mg/kg sodium pentobarbitone. A total of 2 ml of serum was collected for biomarker assays. The left and right femora were dissected for bone biomechanical analysis and micro-CT analysis, respectively. The left proximal tibial metaphysis (PTM) was cut into decalcified 5 μm sections for immunohistochemistry (IHC) analysis. The right PTM was used for reverse transcription-quantitative PCR (RT-qPCR) analysis.

Serum biomarker assay. Blood samples (5 ml) were processed as previously described (12). Briefly, the serum was separated by centrifugation at 1,000 x g at room temperature for 15 min and stored at -80˚C. The serum lipids (TC, TG and LDL-C) and serum bone formation markers (OCN and CTX-I) were determined using ELISA kits (Nanjing Jiangcheng Biological Bioengineering), according to the manufacturer's protocol.

Biomechanical analysis of rat femora. In the biomechanical bone test, the left femora from each group of rats was placed in 70% ethanol for preservation at room temperature. The liquid attached to the femur was blotted with medical gauze, followed by the experiment of bone biomechanical measurement. The Lloyd LR5K Plus Bone Biomechanical Detection system (LLOYD Instruments Ltd.; AMETEK Test & Calibration Instruments) was used to perform a three-point bending test to analyze the biomechanical properties of the femora. The left femur of each group of rats was placed into the machine at a loading speed of 2 mm/min and a span load of 20 mm. Each biomechanical property, including maximum load, breaking load, elastic load and the bone rigidity coefficient, was plotted corresponding to

Table I. Treatments and dosages use for the animal experimental design.

Group	Treatment and dosage
Control	Normal control rats were administered a high-sugar high-fat diet +1% sodium carboxymethyl cellulose (5 ml/kg) daily by oral gavage
T2DM	Diabetic control rats were administered a high-sugar high-fat diet +1% sodium carboxymethyl cellulose (5 ml/kg) daily by oral gavage +3% STZ (40 mg/kg) by intraperitoneal injection
T2DM + Cur	Diabetic rats were administered a high-sugar high-fat diet +3% STZ (40 mg/kg) by intraperitoneal injection + Cur (110 mg/kg/day) orally
T2DM + Cal	Diabetic rats were administered a high-sugar high-fat diet +3% STZ (40 mg/kg) by intraperitoneal injection + Cal (0.045 μg/kg/day) orally
T2DM + Met	Diabetic rats were administered a high-sugar high-fat diet +3% STZ (40 mg/kg) by intraperitoneal injection + Met (200 mg/kg/day) orally

A total of 50 Sprague-Dawley rats were divided into five groups (n=10/group). T2DM, type 2 diabetes mellitus; STZ, streptozotocin; Cur, curcumin; Cal, calcitriol; Met, metformin.

the load-displacement curve and calculated according to the respective equations that reflect bone stiffness (18).

Determination of bone microstructure in rat femora. The metaphyses of the right femurs were measured using a micro-CT (CT-40; Scanco Medical). The specific parameters to test micro-CT parameters were as follows: X-line energy 70 kVP, 114 μA, 500 sheet of 700-nm slices and 30 min scan time. Following the scan, 1.0 mm growth plates were selected from left distal femora. The 3D reconstruction of the femora were constructed according to the following conditions: A thickness of 3.0 mm bone was considered as the cancellous bone region of interest to draw a reconstruction line (19) for bone reconstruction and quantitative image analysis was performed to extract information using the software supplied with the micro-CT (version 4; SCANCO Medical AG). The physical parameters examined were as follows: Bone volume fraction (bone volume/total volume; BV/TV), connection density (Conn.D), trabecular number, (Tb.N), trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th).

RT-qPCR analysis. Gene expression was analyzed using RT-qPCR. Bone tissue from the right tibia (100 mg) was weighed and RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. RT was performed to synthesize cDNA using a kit (cat. no. 6110A; Takara Bio, Inc.), according to the manufacturer's protocol. RT-qPCR was conducted using a SYBR green kit (cat. no. RR420A; Takara Bio, Inc.) on a QuantStudio 12K Flex RT PCR System (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 30 sec; 40 cycles of 95˚C for 5 sec, 60˚C for 45 sec, and the melting curve analysis at 95˚C for 5 sec and 60˚C for 60 sec. The primers used are listed in Table II. β-actin was used as the reference gene. The $2^{-\Delta\Delta Cq}$ method was used to quantify the mRNA expression. All data are presented as relative to the control group.

Table II. Primers used in reverse transcription-quantitative PCR.

Gene	Primer sequences (5'-3')
TGFβ1	Forward: CCATGACATGAACCGACCCT
	Reverse: CCGGGTTGTGTTGGTTGTAG
TβRI	Forward: GGCTCTGCTTTGTCTCTGTCAC
	Reverse: GGTCCTCTTCATTTGGCACTC
TβRII	Forward: GAAGTCTGTGTGGCTGTATGGAG
	Reverse: GCGGTAGCAGTAGAAGATGATG
Smad2	Forward: GCAGGTGGTGGAGAACAGAAT
	Reverse: CCGTATTTGCTGTACTCAGTCCC
Smad3	Forward: CAG GAGGAGAAGTGGTGCGA
	Reverse: TGGTGTTCACGTTCTGCGTG
β-actin	Forward: GACCGCAACAACGCAATCTATGAC
	Reverse:TGCTCCACAGTTGACTTGAATCTCTG

TGFβ1, transforming growth factor β1; TβRI, type I TGFβ receptor I; TβRII, type II TGFβ receptor I.

IHC. Left tibia specimens were fixed in 4% paraformaldehyde for 12 h at room temperature and decalcified for 4 weeks and embedded in paraffin. Sections (5 μm) were placed in a drying oven at 60˚C for 3 h, dewaxed and hydrated, and then washed with 1X PBS solution three times. After 5 min, the tissue specimens were placed in citrate buffer (pH 6.0) at 60˚C for 30 min for antigen retrieval, followed by washing with 1X PBS solution three times for 5 min. Specimens were then incubated with smad2/3 and p-smad2/3 (dilution, 1:200) at 4˚C overnight, followed by incubation with the corresponding secondary antibody (Goat anti-mouse; 1:20,000; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) at room temperature for 2 h. DAB staining was applied for 30 sec at

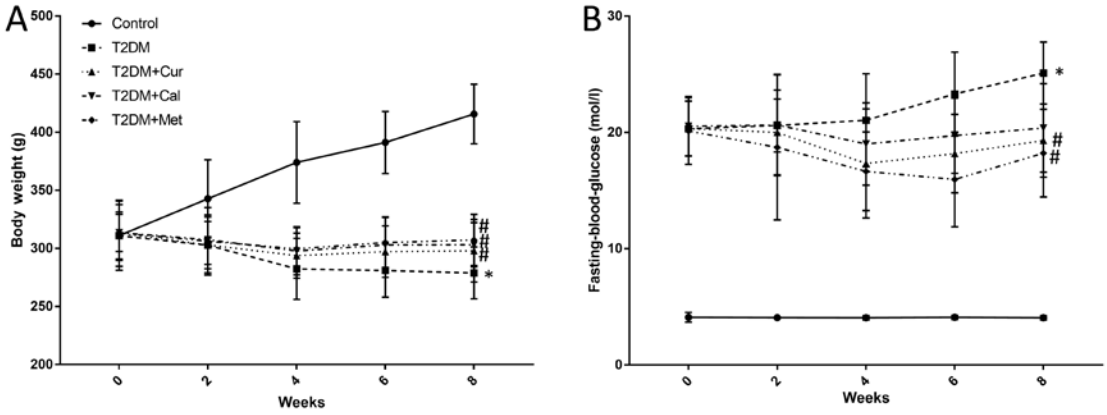

Figure 1. Cur increases body weight and decreases blood glucose levels in T2DM rats. Cur, Cal and Met significantly (A) increased body weight compared with the T2DM group; Cur and Met (B) significantly decreased fasting blood glucose compared with the T2DM group. *P<0.05 vs. controls; #P<0.05 vs. T2DM. T2DM, type 2 diabetes mellitus; cur, curcumin; cal, calcitriol; met, metformin.

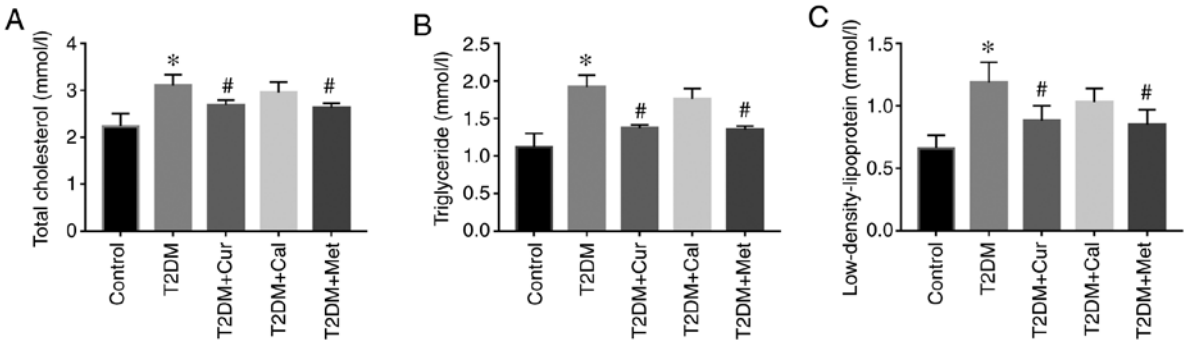

Figure 2. Cur decreases lipid glucose levels in T2DM rats. Cur and Met ameliorated the dysregulated lipid levels in T2DM rats by significantly decreasing serum (A) total cholesterol, (B) triglyceride and (C) low-density lipoprotein levels. *P<0.05 vs. controls; #P<0.05 vs. T2DM. T2DM, type 2 diabetes mellitus; cur, curcumin; cal, calcitriol; met, metformin.

room temperature and hematoxylin counterstaining for 2 min at room temperature, followed by drying and mounting. The stained sections were observed under a light microscope at a magnification of x200 and three fields of view from each bone tissue section were imaged with a Nikon Eclipse E400 camera (Nikon Corporation) with Picture Frame software (E400; Nikon Corporation). The images were analyzed and measured with Image-ProPlus software (version 6.0; Media Cybernetics, Inc.) to determine the mean optical density of the positive area of each specimen, as previously described (20).

Statistical analysis. Data are presented as mean ± standard deviation and were analyzed using SPSS software for Windows (version 16.0; SPSS, Inc.). All experiments were performed in triplicate. Statistical differences between groups were analyzed using one-way ANOVA and Tukey's honest significant difference post-hoc test. Mixed ANOVA was used to compare body weight and glucose level. P<0.05 was considered to indicate a statistically significant difference.

Results

Cur increases body weight and decreases lipid and blood glucose levels in T2DM rats. After the T2DM rat model was established, weight were measured once weekly every 8 weeks. Success of the model was determined by analyzing the body weight and FBG. According to the results, the weight of the rats in the T2DM group was significantly decreased compared with controls (P<0.05) and Cur, Cal and Met increased the body weight of T2DM rats compared with the T2DM group; however, there was no significance between these groups (Fig. 1A). FBG levels in T2DM rats were increased compared with controls (P<0.05), while Cur and Met significantly decreased FBG level compared with the T2DM group. However, there was no significant difference in the levels of of Cal on FBG compared with the T2DM group (P<0.05; Fig. 1B).

At the end of the experiments, serum lipid levels were determined and the results demonstrated that TC, TG and LDL-C were significantly increased following a high-fat, high-sugar diet in T2DM rats compared with controls (P<0.05; Fig. 2). Following treatment with Cur and Met, lipid levels significantly decreased compared with the T2DM group (P<0.05). Furthermore, no significant difference was reported following Cal treatment. These results indicated that Cur may regulate dysregulated lipid and glucose levels and that Met had similar effects.

Cur increases OCN and decreases CTX-I levels. Bone formation marker OCN and bone turnover marker CTX-I levels in serum were measured using respective ELISA kits. The results

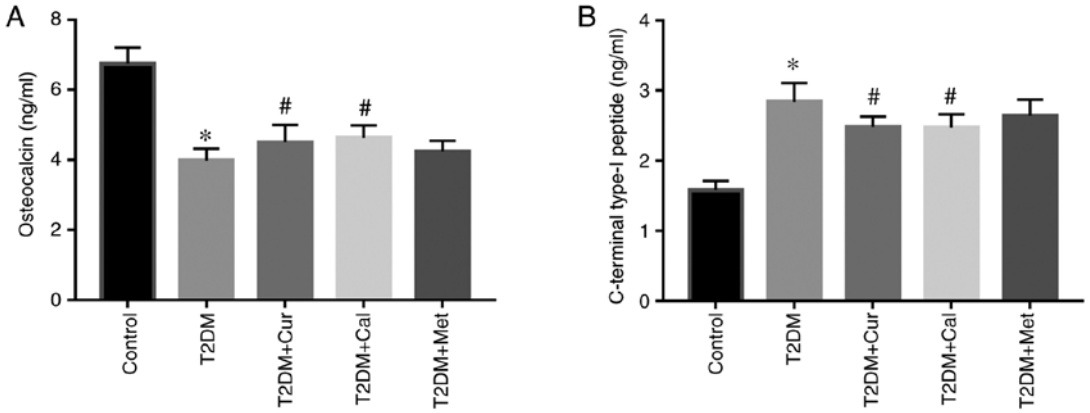

Figure 3. Cur increases OCN and decreases CTX-I levels. Cur and Cal significantly (A) increased the bone formation marker osteocalcin and (B) decreased the bone turnover marker C-terminal type-I peptide in the serum of T2DM rats. *P<0.05 vs. controls; #P<0.05 vs. T2DM. T2DM, type 2 diabetes mellitus; cur, curcumin; cal, calcitriol; met, metformin.

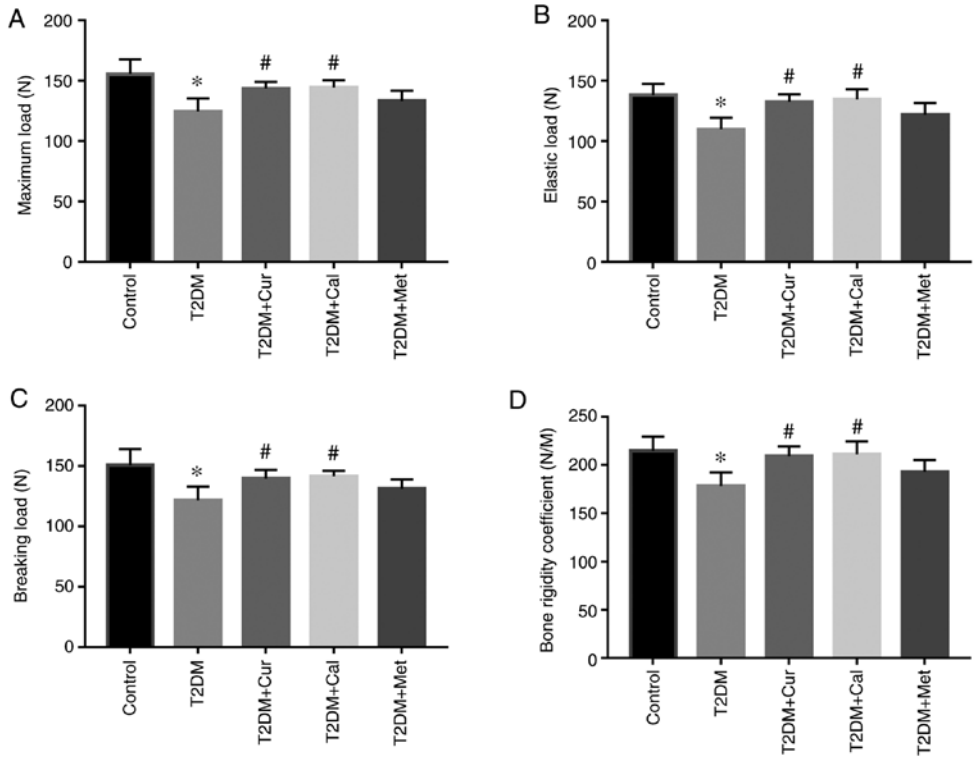

Figure 4. Cur and Cal reversed damaged bone biomechanical properties in T2DM rats. (A) Maximum load, (B) elastic load, (C) breaking load and (D) bone rigidity coefficient were evaluated. *P<0.05 vs. controls; #P<0.05 vs. T2DM. T2DM, type 2 diabetes mellitus; cur, curcumin; cal, calcitriol; met, metformin.

indicated lower bone formation and higher bone absorption activity in T2DM rats compared with controls (P<0.05). However, Cur and Cal reversed this effect by significantly increasing OCN and decreasing CTX-I levels compared with the T2DM group (P<0.05; Fig. 3). There was no significant effect of Met on OCN and CTX-I level compared with the T2DM group.

Cur improves bone biomechanical properties and preserves bone microarchitecture in T2DM rats. To assess the role of Cur in T2DM rat bones, the biomechanical properties of the femur were examined. The results demonstrated that elastic load, breaking load, maximum load and the bone rigidity coefficient of the T2DM group were significantly lower compared with controls (P<0.05; Fig. 4). Moreover, Cur- and Cal-treated groups had significantly improved biomechanical properties compared with the T2DM group. There was no significant effect of Met on biomechanical properties compared with the T2DM group.

Subsequently, micro-CT was used to create representative 3D reconstruction images to examine the trabecular microarchitecture of rat femora (Fig. 5A). Microarchitecture parameters (BV/TV, Conn.D, Tb. N, Tb and Th) in T2DM rats were significantly decreased compared with controls (P<0.05; Fig. 5B), indicating that the high-fat, high-sugar diet was detrimental to rat bone and that the T2DM rat model was successfully established.

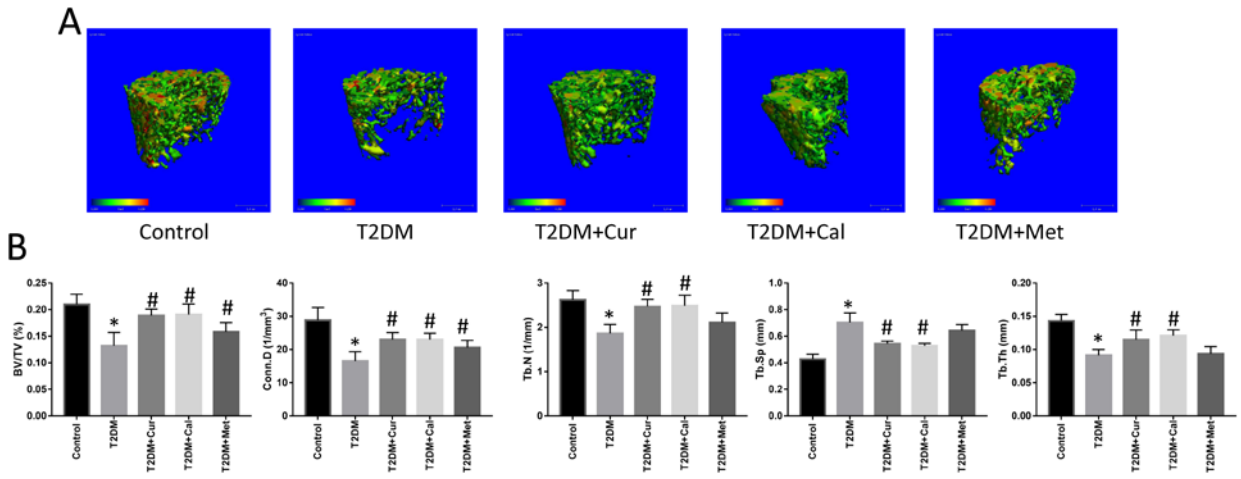

Figure 5. Cur and Cal preserved bone microarchitecture in T2DM rats. (A) 3D reconstruction images of the trabecular microarchitecture of rat femora. (B) Parameters of bone microarchitecture. *P<0.05 vs. controls; #P<0.05 vs. T2DM. T2DM, type 2 diabetes mellitus; cur, curcumin; cal, calcitriol; met, metformin; BV/TV, bone volume fraction; Conn.D, connection density; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness.

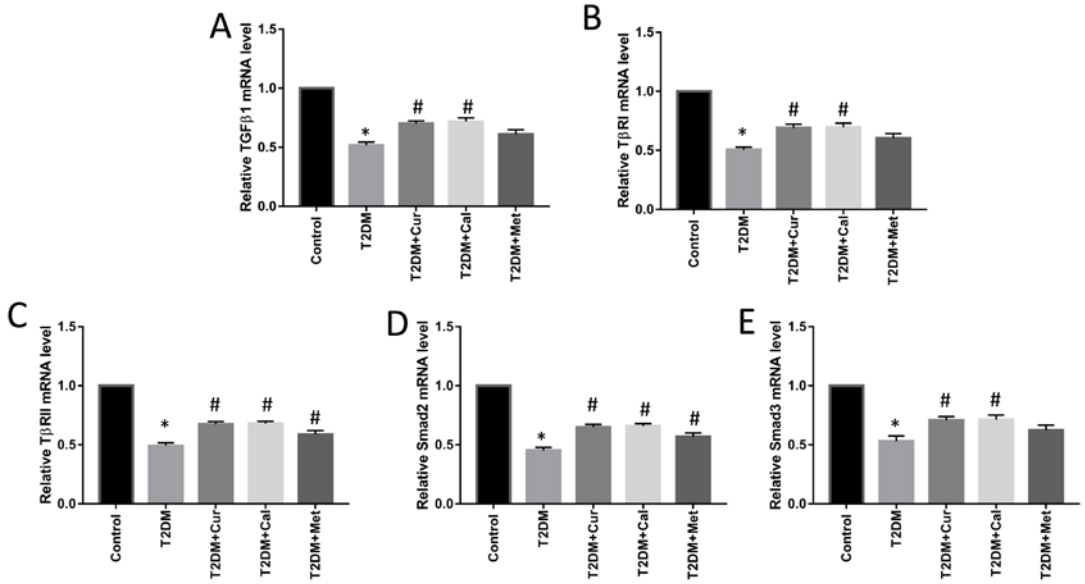

Figure 6. Bone-protective effects of Cur are mediated via the TGFβ/Smad2/3 pathway. Cur and Cal increased (A) TGFβ1, (B) TβRI, (C) TβRII, (D) Smad2 and (E) Smad3 mRNA expression levels in T2DM rat bone. *P<0.05 vs. controls. #P<0.05 vs. T2DM. TGFβ1, transforming growth factor β1; TβRI, type I TGFβ receptor I; TβRII, type II TGFβ receptor I; T2DM, type 2 diabetes mellitus.

Following treatment with Cur, the parameters BV/TV, Conn.D, Tb.N and Tb.Th were significantly increased, while Tb.Sp was decreased compared with the T2DM group (P<0.05). Cal exerted similar effects to Cur in terms of bone biomechanical properties and bone microarchitecture. However, Met did not exhibit any significance between groups.

Bone-protective effects of Cur are mediated via the TGFβ/Smad2/3 pathway. mRNA levels of TGFβ1, TβRI, TβRII and Smad2/3 were determined using RT-qPCR to examine the mechanism of action of Cur. The mRNA levels of TGFβ1, TβRI, TβRII and Smad2/3 in T2DM group were significantly decreased compared with controls (P<0.05) and Cur and Cal significantly increased TGFβ1, TβRI, TβRII and Smad2/3 mRNA expression levels in the right PTM compared with the T2DM group (P<0.05; Fig. 6) and Met significantly increased TβRII and Smad2 mRNA expression levels compared with the T2DM group (P<0.05; Fig. 6)

Furthermore, IHC was performed to evaluate Smad2/3 and p-Smad2/3 protein expression levels. The results indicated that Cur significantly increased Smad2/3 expression and promoted Smad2/3 phosphorylation (Fig. 7). These results suggested that may Cur protect bone via TGFβ/Smad2/3 signaling pathway activation.

Discussion

The aim of the current study was to comprehensively assess the effect of Cur on osteoporosis in T2DM rats by observing 3D structural diagrams of bone microstructures, and evaluating

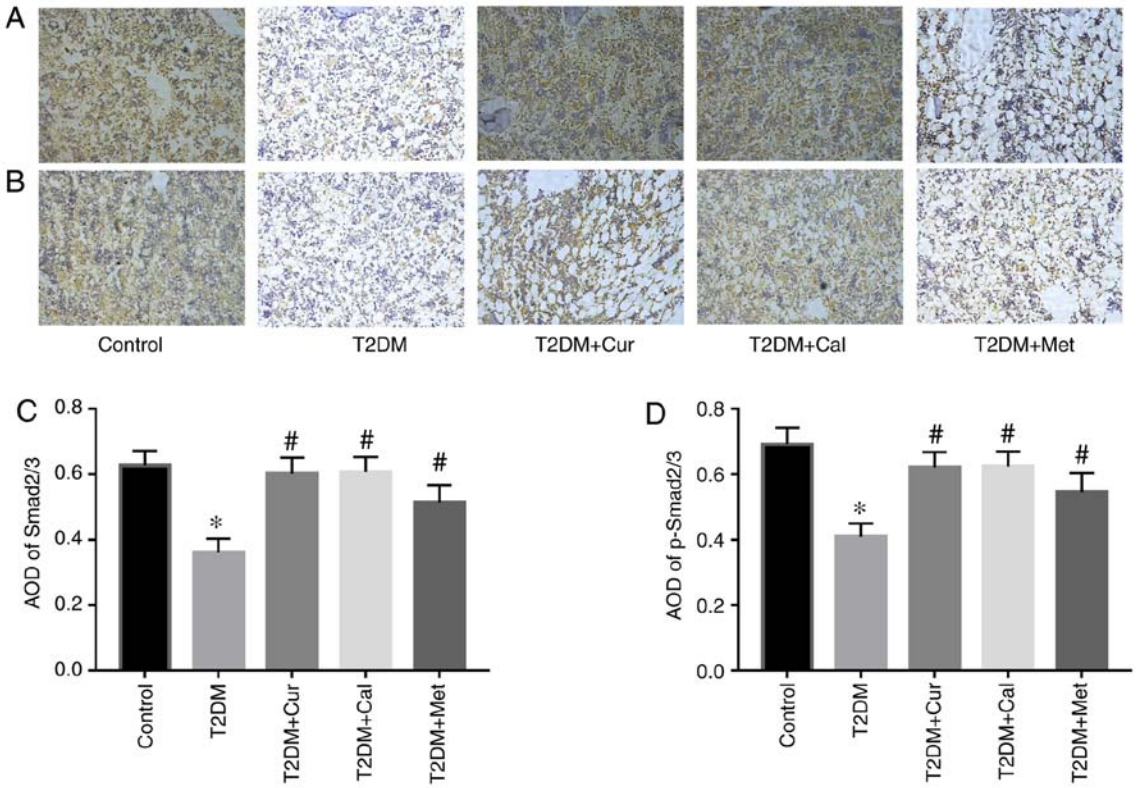

Figure 7. Cur, Cal and Met increased Smad2/3 expression and promoted Smad2/3 phosphorylation as detected by immunohistochemistry. Representative images of (A) Smad2/3 and (B) p-Smad2/3. Magnification, x100. Mean optical density of (C) Smad2/3 and (D) p-Smad2/3. *P<0.05 vs. controls; #P<0.05 vs. T2DM. T2DM, type 2 diabetes mellitus; cur, curcumin; cal, calcitriol; met, metformin; AOD, average optical density; p-, phosphorylated.

bone microstructure, bone biomechanics, serum bone conversion metabolism, blood glucose and blood lipid indicators. Additionally, the TGFβ/Smads signaling pathway was used to provide a theoretical basis for the mechanism of action via which Cur acts in the prevention and treatment of osteoporosis in T2DM.

Recently, it has been reported that certain herbal extracts may prevent bone loss and protect against diabetes-induced bone microarchitecture disruption (21,22). Cur is an extract from the roots of the genus Curcuma and previous studies have revealed that it can reduce inflammation and oxidative stress in a diabetic rat model, improve islet cell function and reduce blood glucose levels (23,24). In addition, Cur may improve diabetes-induced damage of the retina, heart, kidney and other organs, thus demonstrating promising therapeutic effects against diabetic complications (25,26). Furthermore, Cur serves a key role in the metabolism of bone reconstruction (27).

In the present study, the role of Cur in a T2DM rat model and its underlying mechanism of action were investigated. The T2DM rat model was successfully established using a high-sugar, high-fat diet combined with low dose STZ (28). After establishing a successful model, differences in body mass were observed among the five groups of rats. The body mass of the T2DM, T2DM + Cur, T2DM + Cal and T2DM + Met groups was lower compared with control group, and this difference persisted until the end of the experiment. After week 8, the body mass of the T2DM + Cur, T2DM + Cal and T2DM + Met groups was higher compared with the T2DM group. Therefore, it was speculated that body mass notably affected bone density and bone mass in the present study.

Epidemiological studies have reported that patients with diabetes often exhibit increased TC, TG and LDL-C levels, which may lead to abnormal lipid metabolism (29). The present results of serum lipid measurement in the T2DM group were consistent with those of previous studies (29,30). Furthermore, the present results demonstrated that dyslipidemia in T2DM rats was alleviated by Cur or Met. This effect may be associated with the ability of Met to activate the 5'AMP-activated protein kinase pathway, inhibit endogenous liver X receptor, reduce the expression of sterol regulatory element-binding protein 1, improve fatty acid metabolism, improve hyperlipidemia and reduce the risk of atherosclerosis (31).

Rat femoral bone mass was evaluated by micro-CT and the parameters of bone morphometry examined included BV/TV, Conn.D, Tb.Sp, Tb.N and Tb.Th. The results demonstrated that BV/TV, Conn.D, Tb.N and Tb.Th were lower in the T2DM group compared with controls, while Tb.Sp was increased; this indicated that a high-sugar, high-fat diet combined with low doses of STZ damaged bone microarchitecture. The 3D reconstruction was also in line with this finding, as the results indicated trabecular bone degeneration and cancellous bone loss, which lead to decreased bone mass. Additionally, the biomechanical properties of femora were lower in the T2DM group compared with controls. Therefore, T2DM rats exhibited dysregulated lipid and glucose levels, and disrupted bone biomechanical properties and microarchitecture. Bone mineral density was not evaluated in the present study as the research was focused on the bone microarchitectures.

Insulin can directly stimulate osteoblasts, and promote amino acid accumulation and bone collagen and matrix synthesis (32). In patients with diabetes, insulin secretion is maladjusted or dysfunctional, and insulin resistance may lead to osteoporosis (33). CTX-I and OCN levels are representative markers of osteoclast and osteoblast differentiation, respectively (34). When insulin resistance occurs, CTX-I levels increase, leading to bone resorption (35). In the present study, the bone formation marker OCN was decreased and the bone turnover marker CTX-I was increased in the serum of T2DM rats, indicating that bone formation was inhibited and bone resorption was activated, respectively. The results demonstrated that CTX-I levels in the T2DM + Cur group were lower compared with the T2DM group, indicating that Cur suppressed the function of osteoclasts and inhibited bone resorption in diabetic rats. It has been previously reported that Cur improved tartrate-resistant acid phosphatase activity and mRNA expression in diabetic rats, indicating that Cur treatment decreases osteoclast activity and thus inhibits bone resorption and protects bone microstructure (36).

In the present study, following treatment with Cur, lipid and glucose levels improved and Met treatment demonstrated similar results. The hypoglycemic mechanism involved may be associated with the enhancement of antioxidant capacity, immunity and hepatic glucokinase activity, and Cur may serve a role in decreasing lipid levels by increasing the reductase activity of β-hydroxy β-methylglutaryl-coenzyme A, thus increasing the number of LDL receptors in the liver and removing cholesterol from tissues (37). However, the mechanism underlying its hypoglycemic and lipid-lowering properties has not been fully elucidated. In the present study, abnormal glucose and lipid metabolism in T2DM was accompanied by changes in bone ultrastructure. The underlying mechanism may involve the disruption of the dynamic balance of bone remodeling due to disordered glucose and lipid metabolism, and hyperglycemia, resulting in bone absorption that overrides bone formation and leads to bone loss (38). Cur treatment improved bone metabolism and structure, which may be due to the reduction of blood glucose and lipid levels and enhanced bone formation. In addition, the results demonstrated that Cal was unable to regulate lipid and glucose levels. It was also found that the bone formation marker OCN was increased and the bone turnover marker CTX-I decreased by Cur; Cal exerted a similar effect. However, effect of Met on bone formation marker did not significantly increase OCN expression, indicating that the effect of Met on osteoporosis was limited.

Numerous cytokines are involved in bone remodeling, including TGFβ1, which is the primary cytokine associated with this process (39,40). TGFβ1 is secreted by osteoblasts and promotes osteoblast differentiation and inhibits osteoclast activity (41). A previous study reported that T2DM rats exhibited lower TGFβ1 expression when the bone was damaged compared with that at baseline (42). However, increasing TGFβ1 level protects bone as it slows bone loss and delays diabetic damage and may therefore be a promising therapeutic target for the management of T2DOP (43). The current study demonstrated that Cur increased TGFβ1 mRNA expression and promoted Smad2/3 phosphorylation, indicating that the effects of Cur may be mediated via the TGFβ1/Smad2/3 pathway.

The aim of the current study was to identify a natural drug that can simultaneously be used in the treatment of diabetes and osteoporosis. The present study investigated the effect of Cur on lipid and glucose levels, bone biomechanical properties and bone microarchitecture in T2DM rats. Collectively, it was demonstrated that serum lipid and blood glucose dysregulation was ameliorated by Cur. Moreover, the loss of bone mass and the disruption of bone microstructure and biomechanical properties were reversed by Cur. To the best of our knowledge, the present study was the first to demonstrate that Cur protects the bone in T2MD rats via the TGFβ/Smad2/3 pathway *in vivo*. However, the current study had certain limitations. First, the underlying mechanism of Cur requires further investigation. Second, the effect of Cur on the bone mineral density remains unclear. Overall, it was hypothesized that Cur may have a potential clinical application in secondary osteoporosis. However, its poor bioavailability remains the main limitation restricting its application.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Zhaoqing Medical College 2017 Annual University-Level Research Project (grant no. 2017K12), the 2019 Guangdong Medical Research Fund Project (grant no. B2019229), the Science and Technology Million Project Joint Project of Inner Mongolia Medical University [grant no. YKD2018KJBW(LH)082] and the Medical Health Science and Technology Project of Baotou Inner Mongolia (grant no. WSJJ2019029).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL, BZ and SL performed the experiments, analyzed data, participated in study design and wrote the manuscript. YZh, YY and ZB conducted data analysis. YZe and DL designed the study and participated in development and coordination. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments were approved by the Ethics Committee of Zhaoqing Medical College.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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