Oral administration of quercetin inhibits bone loss in rat model of diabetic osteopenia

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A B S T R A C T

Diabetic osteopenia can result in an increased incidence of bone fracture and a delay in fracture healing. Quercetin, one of the most widely distributed flavonoids in plants, possesses antioxidant property and beneficial effect on osteoporosis in ovariectomized mice. All these properties make quercetin a potential candidate for controlling the development of diabetic osteopenia. Therefore, the present study was designed to investigate the putative beneficial effect of quercetin on diabetic osteopenia in rats. Diabetes mellitus was induced by streptozotocin. The diabetic rats received daily oral administration of quercetin (5 mg/kg, 30 mg/kg and 50 mg/kg) for 8 weeks, which was started at 4 weeks after streptozotocin injection. Quercetin at 5 mg/kg showed little effect on diabetic osteopenia, while quercetin at 30 mg/kg and 50 mg/kg could increase the decreased serum osteocalcin, serum alkaline phosphatase activity, and urinary deoxypyridinoline in diabetic rats. In addition, quercetin (30 mg/kg and 50 mg/kg) could partially reverse the decreased biomechanical quality and the impaired micro-architecture of the femurs in diabetic rats. Histomorphometric analysis showed that both decreased bone formation and resorption were observed in diabetic rats, which was partially restored by quercetin (30 mg/kg and 50 mg/kg). Further investigations showed that quercetin significantly lowered the oxidative DNA damage level, up-regulated the total serum antioxidant capability and the activity of serum antioxidants in diabetic rats. All those findings indicate the beneficial effect of quercetin on diabetic osteopenia in rats, and raise the possibility of developing quercetin as potential drugs or an ingredient in diet for controlling diabetic osteopenia.

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1. Introduction

Diabetes mellitus is one of the most frequently encountered clinical problems, which affects more than 250 million people, and has become the fourth leading cause of disease-related death around the world currently. It has been well recognized that diabetes mellitus may result in many complications, including nephropathy, retinopathy, neuropathy and osteopenia. Particularly, osteopenia always lead to an increased incidence of bone fracture, a delay in healing of bone fractures, and affect the quality of life in diabetic patients (Forsén et al., 1999; Herskind et al., 1992; Vestergaard, 2007). Therefore, searching for effective drugs which can control the development of diabetic osteopenia is of great significance for patients with diabetic osteopenia. The mechanisms responsible for diabetic osteopenia have not been clear thus far. It has been reported that hyperglycemia is one of the contributing factors to diabetic osteopenia. High glucose level is capable of influencing osteoblast differentiation, impairing bone formation and inhibiting bone mineralization (Zhen et al., 2010). In addition, hyperglycemia is able to trigger increased oxidative stress (King and Loeken, 2004). The increased oxidative stress has been demonstrated to inhibit osteoblast differentiation and induce osteoblast insults and apoptosis, and has been deemed as an important contributing factor for the incidence of diabetic osteopenia (Bai et al., 2004; Hamada et al., 2009). Therefore, simultaneous control of hyperglycemia and oxidative stress has great potential in controlling the incidence and development of diabetic osteopenia. Quercetin is a naturally occurring flavonoid found in onions and other vegetables, and potentially has beneficial effects on disease prevention. Many special properties of quercetin make it an ideal candidate substance for the prevention and treatment of diabetic osteopenia. It has been reported that quercetin exerts protective effects against β-cell damage in diabetes and is capable of decreasing serum glucose level in diabetic rats (Coskun et al., 2005). In addition, quercetin is a powerful natural antioxidant. It has been reported that quercetin can protect many types of tissue or organs against oxidative damage, including liver (Yokoyama et al., 2009; Yousef et al., 2010), kidney (Yousef et al., 2010) and pancreatic gland (Coskun et al., 2005). Furthermore, quercetin has beneficial effect on bone cells and tissues. Quercetin is capable of increasing alkaline phosphatase...
(ALP) activity in MG-63 osteoblasts (Robaszkiewicz et al., 2007). Further studies show that dietary quercetin can inhibit bone loss in ovariectomized mice (Tsuij et al., 2009), confirming the beneficial effect of quercetin on bone tissue in bone disorders. Taken together, the antioxidant capability of quercetin, its beneficial effect on osteoblast and bone tissue, and its capability of lowering serum glucose level make quercetin a potential candidate for controlling the development of diabetic osteopenia. Therefore, the present study was designed to investigate the putative beneficial effect of quercetin on diabetic osteopenia in diabetic rats.

2. Materials and methods

2.1. Diabetic rat model and quercetin treatment

Eighty male Sprague–Dawley rats weighing 200–220 g were provided by the Animal Center of the Fourth Military Medical University (FMMU), and were assigned to saline treated non-diabetic group (n=10), quercetin-treated non diabetic group (n=30), saline-treated diabetic group (n=10) and quercetin-treated diabetic group (n=30). The saline-treated diabetic and quercetin-treated diabetic rats were intraperitoneally injected with streptozotocin (Sigma, Chemical Co., St Louis, MO, USA) at 100 mg/kg body weight in 100 µL of sterile citrate buffer (pH 4.5) on two consecutive days. Control rats were intraperitoneally injected with citrate vehicle alone. All rats received normal diet and water. Blood samples obtained from the tail vein were obtained to determine the serum glucose level by OneTouch SureStep Plus glucometer (Lifescan, Milpitas, CA, USA), and rats with venous serum glucose levels of over 17 mmol/L were considered diabetic. In quercetin-treated rats, oral administration of quercetin (5 mg/kg, 30 mg/kg and 50 mg/kg) were performed daily through a custom-made stomach tube. In saline-treated rats, equal amount of saline were orally administrated through stomach tubes. Quercetin and saline treatments were started from the fourth weeks after streptozotocin injection and were maintained for 8 weeks.

The body weight and the serum glucose of the animals were recorded weekly during the experimental period. Urine sample was collected from each rat that was individually housed for 24 h in metabolic cages without providing food 1 day before sacrifice. After induction of anesthesia with diethyl ether, blood sample was collected by cardiac puncture, and serum was prepared from each blood sample by centrifugation for 5 min at 850 × g. Urine and serum samples were then stored at −80 °C for biochemical determinations. Femurs were dissected and filled in physiological saline and stored at −20 °C for measurement of total bone mineral content (BMC) and bone mineral density (BMD) by Dual-energy X-ray absorptiometry, trabecular micro-architecture was determined by Microcomputed tomography (micro-CT), and bone biomechanical quality was measured by a three-point bending test. All procedures in the present study were reviewed and approved by the institutional Ethical Committee of the FMMU.

2.2. Assay for serum and urine chemistry

Serum alkaline phosphatase (ALP) concentration was analyzed by standard colorimetric methods using commercial kits (ZhongSheng BeiKong Bio-technology and Science, China). Serum osteocalcin concentration was determined using an ELISA kit (Quidel Corporation, San Diego, CA, USA). Urinary creatinine (Cr) concentration was assayed using an automatic biochemical analyzer (Roche Diagnostics, Switzerland). Urinary deoxypyridinoline (DPD) concentration was assayed using a rat DPD ELISA kit (Quidel Corporation, San Diego, CA, USA). Urinary excretion of DPD was expressed as the ratio to Cr concentration (urinary DPD/Cr).

2.3. Dual-energy X-ray absorptiometry

The bone mineral content (BMC) and bone mineral density (BMD) of the right femur were determined using Lunar Prodigy Advance by Dual-energy X-ray absorptiometry (GE Healthcare, USA), which has been equipped with appropriate software for bone density assessment in small laboratory animals as reported previously (Zhang et al., 2009). BMD was calculated by BMC of the measured area.

2.4. Micro-CT analysis

The trabecular bone mass and microarchitecture of the right femurs were analyzed using a microcomputer tomography (Micro-CT) scanner (GE healthcare, Madison, WI, USA). The distal femur was placed in a sample tube (20 mm in diameter), which was positioned perpendicularly to the scanning axis. The total reconstructed height was restricted to 12 mm. The scanning started from the plane of the lower growth plate and extended proximally for 350 slices (16 µm/slice). A volume of interest (VOI) was selected as a region which started at the level of 25 slices away from the lower growth plate, included 100 slices. The parameters of trabecular bone mass and microarchitecture were quantitatively analyzed with the MicroView program (GE Healthcare, Madison, WI, USA). The MicroCT quantitative parameters includes: trabecular number (Tb.N), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), connectivity density (Conn.D), structure model index (SMI), bone volume with total volume as referent (BV/TV), cortical thickness (C.Th), and cortical bone area (C.Ar).

2.5. Three-point bending test

The biomechanical property of the left femurs was determined by a three-point bending test. The frozen left femurs were thawed at room temperature for 1 h, and then were immobilized on a fixing supporter in a biomechanical testing system (AGS-10kNC, Shimadzu, Kyoto, Japan) with a fixed distance of 20 mm between two loading points. Compressive load was applied to the femurs at a constant rate of 2 mm/min until fracture was observed. The inner and outer diameters and height of the femur at the point of fracture were recorded with a vernier caliper. Data of the force and displacement were automatically recorded by a computer which was connected to the testing machine. The indices we determined included the followings: maximum load (the maximum tensile load that the femur can sustain before fracture), stiffness (slope of the linear part of the curve representing elastic deformation), and energy absorption (area under the load-deformation curve). Elastic modulus (E) was calculated according to the formula (Turner and Burr, 1993),

\[ E = FL^3/48DI \]

where F is the maximum load, L is the distance between supporting points, D is the displacement, I is the moment of inertia of the cross-section in relation to the horizontal axis.

2.6. Histomorphometric analysis

Rats in each group received intraperitoneal injection of 20 mg/kg calcin 12 and 4 days before sacrifice. The right proximal tibias were obtained and used as undecalciﬁed specimens for dynamic bone histomorphometry analysis. Samples were ﬁxed in 10% phosphate-buffered formalin for 24 h, dehydrated in a vacuum desiccator with graded ethanol, then deﬁbriﬁed in xylene and embedded in methyl methacrylate. Slides with 10 µm-thicknesses were prepared with a hard tissue microtome (Leica SP 2155, Germany). The slides were processed for H-E staining and tartrate-resistant acid phosphatase staining. The bone histomorphometric analysis followed the protocols reported
peroxidase, glutathione S-transferase (GST) was determined in the present study, and the serum total antioxidant capacity was calculated as mmol/L serum.

2.7. Serum total antioxidant capacity

Serum total antioxidant capacity was determined using a commercial kit (Abcam, USA), following the manufacturer's instructions. This assay was based on the extent of suppression of ABTS (2, 2'-Azino-di-[3-ethylbenzthiazoline sulfonate]) oxidation in the presence of metmyoglobin and H₂O₂. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid was provided as a standard antioxidant in the present study, and the serum total antioxidant capacity was calculated as mmol/L serum.

2.8. Serum antioxidant activity

Serum activity of superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione S-transferase (GST) was determined in the present study. In brief, serum SOD activity was determined by the method of Kakkar in which the inhibition of formation of NADPH-phenazine methosulfate nitroblue tetrazolium formazan was measured spectrophotometrically at 560 nm (Kakker et al., 1984). Serum catalase activity was assayed colorimetrically using dichromate-acetic acid reagent as described by Sinha (Sinha, 1972). Serum glutathione peroxidase activity was assayed by the method of Rotrucketal, which based on the reaction between glutathione remaining after the action of glutathione peroxidase and 5, 5'-dithiobis-2-nitrobenzoic acid to form a complex that absorbs maximally at 412 nm (Rotruck et al., 1973). Serum GST activity was spectrophotometrically determined by the method of Habig et al. in which dichloro-2, 4-dinitrobenzene was used as the substrate (Habig et al., 1974).

2.9. Assessment of systemic oxidative stress

The concentration of urinary 8-hydroxydeoxyguanosine (8-OHDG) has been considered as a sensitive indicator of oxidative DNA damage, and was determined to evaluate the extent of oxidative stress in the animals in the present study. After 12 weeks of streptozotocin treatment, a 24 h-urine collection from each rat using metabolic cages. The urine was kept at −20 °C until the concentrations of 8-OHDG were measured using a commercial ELISA assay kit (JaICA, Tokyo, Japan).

2.10. Statistical analysis

All data were expressed as the mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to compare mean values using SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA). If there was a significant overall difference among groups, then Tukey’s post hoc test was used to make pair-wise comparisons. Values of p<0.05 were considered statistically significant.

3. Results

3.1. Body weight and serum glucose

The initial body weights of the rats were similar between groups. The body weights of the non-diabetic rats which received normal diet gradually increased over time. The body weights of the streptozotocin induced diabetic rats were significantly lower than that of the non-diabetic rats (P<0.05, Fig. 1A). The body weights of the diabetic rats which received quercetin at 5 mg/kg were in similar range to that of the saline treated diabetic rats (P>0.05, Fig. 1A). The body weights of the diabetic rats which received quercetin at 30 mg/kg and 50 mg/kg were significantly higher than that of the saline treated diabetic rats (P<0.05, Fig. 1A).

Oral administration of quercetin showed little effect on serum glucose in non-diabetic rats. The serum glucose levels of the saline treated diabetic rats were significantly higher than that of the saline treated non-diabetic rats (P<0.05, Fig. 1B). Treatment with quercetin at 30 mg/kg and 50 mg/kg significantly lowered the serum glucose level in diabetic rats (P<0.05, Fig. 1B). Quercetin at 5 mg/kg showed little effect on serum glucose level in diabetic rats (P>0.05, Fig. 1B).

3.2. Serum and urinary chemistry

Twelve weeks after streptozotocin treatment, serum osteocalcin and ALP activity, two bone formation markers, were significantly inhibited in saline treated diabetic rats compared to that of saline treated non-diabetic rats (P<0.05, Table 1). Quercetin at 30 mg/kg and 50 mg/kg significantly increased the serum osteocalcin and ALP levels in diabetic rats, while quercetin at 5 mg/kg had little influence on serum osteocalcin and ALP levels in diabetic rats. Urinary DPD/Cr ratio, a bone resorption marker, was significantly lower in saline treated diabetic rats compared to that of the saline treated non-diabetic rats (p<0.05, Table 1). The urinary DPD/Cr ratio in diabetic rats was significantly increased by quercetin at 30 mg/kg and 50 mg/kg (P<0.05, Table 1). The urinary DPD/Cr ratio in diabetic rats treated with quercetin at 5 mg/kg was in the similar range to that in saline treated diabetic rats. Treatment with quercetin had little effect on serum osteocalcin, serum ALP and urinary DPD/Cr ratio in non-diabetic rats.
3.3. Biomechanical quality of the femurs

Twelve weeks after streptozotocin injection, the maximal load (Fig. 2A), stiffness (Fig. 2B), energy absorption (Fig. 2C) and Young modulus (Fig. 2D) of the femurs in saline treated diabetic rats were significantly lower than that in saline treated non-diabetic rats ($P < 0.05$, Fig. 2A, B, C, D). Quercetin at 30 mg/kg and 50 mg/kg significantly improved the mechanical strength of femurs in diabetic rats. The maximal load, stiffness, energy absorption and Young modulus of the femurs in quercetin treated diabetic rats (30 mg/kg and 50 mg/kg) were significantly higher than those in saline-treated diabetic rats ($P < 0.01$, Fig. 2A, B, C, D). Quercetin at 5 mg/kg showed little effect on the mechanical strength of femurs in diabetic rats. Quercetin had little effect on the biomechanical quality of the femurs in non-diabetic rats.

3.4. BMD and BMC of the femurs

No significant difference in femur BMC was found among saline and quercetin-treated diabetic rats ($P > 0.05$, Fig. 3A). Whereas, the femur BMD in saline treated diabetic rats was significantly lower than that in saline treated non-diabetic rats. Treatment with quercetin at 30 mg/kg and 50 mg/kg significantly increased the femur BMD in diabetic rats, while quercetin at 5 mg/kg had little influence on the femur BMD in diabetic rats ($P > 0.05$, Fig. 3B). Quercetin treatment had little effect on the BMD and BMC of femurs in non-diabetic rats.

3.5. MicroCT analysis

Micro CT was adopted to investigate the changes of microarchitecture in distal femurs. Diabetic rats showed a significant decrease in $Tb·Th$, $Tb·N$, $BV/TV$, Conn.D, Ct·Th and Ct·Ar, and an increase in $Tb·Sp$ and SMI ($P < 0.01$, Table 2) compared to the saline treated non-diabetic rats. The above indices in diabetic rats treated with quercetin at 5 mg/kg were in the similar range to that in the saline treated diabetic rats. Quercetin at 30 mg/kg and 50 mg/kg significantly improved the trabecular bone mass loss and microarchitecture deterioration in diabetic rats (Table 2). The values of $Tb·Th$, $Tb·N$, $BV/TV$, Conn.D, Ct·Th and Ct·Ar were significantly higher, and the values of $Tb·Sp$ and SMI were significantly lower in diabetic rats treated with quercetin (30 mg/kg and 50 mg/kg) than that in saline treated diabetic rats. Quercetin showed little effect on the Micro-CT parameters in non-diabetic rats.

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-diabetic rats</th>
<th>Diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Quercetin (50 mg/kg)</td>
</tr>
<tr>
<td>S-ALP (U/L)</td>
<td>124.6 ± 11.8</td>
<td>128.4 ± 12.2</td>
</tr>
<tr>
<td>S-OC (mmol/L)</td>
<td>8.32 ± 0.75</td>
<td>8.42 ± 0.69</td>
</tr>
<tr>
<td>U-DPD/ Cr (mmol/mmol)</td>
<td>48.17 ± 5.33</td>
<td>46.73 ± 4.73</td>
</tr>
</tbody>
</table>

* $P < 0.05$.  
$^a$ $P < 0.01$ vs. saline-treated diabetic group.  
$^b$ $P < 0.05$.  
$^d$ $P < 0.01$ vs. saline-treated non-diabetic group.

Fig. 2. Effects of 8-week treatment of quercetin on biomechanical indices of femurs. The maximum load (A), stiffness (B), energy absorption (C), and elastic modulus (D) in each group was shown. Values are all expressed as mean ± S.D. ($n = 10$). $^* P < 0.05$ vs. saline-treated diabetic group; $^a P < 0.05$, $^b P < 0.01$ vs. saline-treated non-diabetic group.
**3.6. Histomorphometric analysis**

A remarkable decrease in BFR/BS, MS/BS and MAR, which are indicators of bone formation, was found in saline treated diabetic rats compared to those in non diabetic rats (P<0.05, Fig. 4A, B, C), suggesting that bone formation was inhibited in diabetes mellitus. In addition, Oc.S/BS was found to be significantly lower in diabetic rats (P<0.01, Fig. 4D), indicating that bone resorption was inhibited either in diabetes mellitus. Treatment with quercetin (30 mg/kg and 50 mg/kg) significantly increased BFR/BS, MS/BS, MAR and Oc.S/BS in diabetic rats (P<0.01, Fig. 4), while quercetin at 5 mg/kg had little influence on the above parameters in diabetic rats (P>0.05, Fig. 4). Quercetin treatment had little effect on BFR/BS, MS/BS, MAR and Oc.S/BS in non-diabetic rat (P>0.05, Fig. 4).

**Table 2**

Micro-CT parameters of the trabecular bone in the distal femur region.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-diabetic</th>
<th>Quercetin (50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th-N (µm)</td>
<td>4.22±0.31</td>
<td>4.17±0.29</td>
</tr>
<tr>
<td>Th-th (µm)</td>
<td>0.11±0.02</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>Th-Sp (µm)</td>
<td>0.13±0.011</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>0.45±0.052</td>
<td>0.46±0.037</td>
</tr>
<tr>
<td>SMI (#)</td>
<td>0.53±0.047</td>
<td>0.51±0.038</td>
</tr>
<tr>
<td>Conn.D (#/mm³)</td>
<td>56.61±6.72</td>
<td>54.72±6.15</td>
</tr>
<tr>
<td>Ct-Th (µm)</td>
<td>532.7±47.16</td>
<td>534.27±45.29</td>
</tr>
<tr>
<td>Ct-Ar (mm²)</td>
<td>7.41±0.63</td>
<td>7.38±0.56</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic</th>
<th>Quercetin (5 mg/kg)</th>
<th>Quercetin (30 mg/kg)</th>
<th>Quercetin (50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th-N (µm)</td>
<td>2.63±0.24 ^d</td>
<td>2.63±0.24 ^d</td>
<td>3.51±0.02 ^bc ^d</td>
<td>3.76±0.35 ^bc ^d</td>
</tr>
<tr>
<td>Th-th (µm)</td>
<td>0.071±0.006 ^d</td>
<td>0.071±0.006 ^d</td>
<td>0.086±0.009 ^ac</td>
<td>0.093±0.011 ^ac</td>
</tr>
<tr>
<td>Th-Sp (µm)</td>
<td>0.33±0.037 ^d</td>
<td>0.33±0.037 ^d</td>
<td>0.25±0.019 ^bc</td>
<td>0.17±0.018 ^bc</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>0.24±0.019 ^d</td>
<td>0.24±0.019 ^d</td>
<td>0.36±0.035 ^bc</td>
<td>0.38±0.027 ^bc</td>
</tr>
<tr>
<td>SMI (#)</td>
<td>1.22±0.096 ^d</td>
<td>1.22±0.096 ^d</td>
<td>0.85±0.073 ^bc</td>
<td>0.79±0.062 ^bc</td>
</tr>
<tr>
<td>Conn.D (#/mm³)</td>
<td>36.28±3.74 ^d</td>
<td>36.28±3.74 ^d</td>
<td>43.17±3.93 ^ac</td>
<td>47.25±4.18 ^bc</td>
</tr>
<tr>
<td>Ct-Th (µm)</td>
<td>313.4±29.01 ^d</td>
<td>313.4±29.01 ^d</td>
<td>380.7±35.14 ^d</td>
<td>426.9±37.52 ^ac</td>
</tr>
<tr>
<td>Ct-Ar (mm²)</td>
<td>3.68±0.25 ^d</td>
<td>3.68±0.25 ^d</td>
<td>4.95±0.46 ^bc ^d</td>
<td>5.28±0.51 ^bc</td>
</tr>
</tbody>
</table>

^a^ P<0.05.
^b^ P<0.01 vs. saline-treated diabetic group.
^c^ P<0.05.
^d^ P<0.01 vs. saline-treated non-diabetic group.

4. Discussion

The present study investigated the effect of quercetin on diabetic osteopenia in rats. We found that oral administration of quercetin was capable of reversing the decreased serum osteocalcin, serum ALP activity, and urinary DPD/Cr ratio which were found in diabetic rats. Quercetin at 5 mg/kg showed little effect on diabetic osteopenia, while oral administration of quercetin (30 mg/kg and 50 mg/kg) could partially reverse the decreased biomechanical quality, and the impaired micro-architecture of the femurs in diabetic rats. Further investigations showed that quercetin (30 mg/kg and 50 mg/kg) significantly lowered the urinary 8-hydroxydeoxyguanosine (8-OHdG) level (a sensitive indicator of oxidative DNA damage), up-regulated the total serum antioxidant capability and the activity of serum antioxidants in diabetic rats. All those findings indicate the beneficial effect of quercetin on osteopenia induced by diabetes in rats, and raise the possibility of developing quercetin as a potential therapeutic drug for diabetic osteopenia.

The streptozotocin induced type I diabetes mellitus in rats is a well characterized animal model for metabolic and pharmacological investigations (Lenzen, 2008; Suzuki et al., 2003). It has been reported that injection of streptozotocin was capable of causing prompt destruction of pancreatic β-cells, and resulting in remarkable hyperglycemia and weight loss in rats (Lenzen, 2008). The hyperglycemia induced by streptozotocin could lead to decreased bone density, decreased biomechanical quality, and impaired micro-architecture in femurs in

Fig. 3. Effects of 8-week treatment of quercetin on bone mineral content (BMC, A) and bone mineral density (BMD, B) in diabetic rats. Values are all expressed as mean±S.D. (n = 10). *P<0.05 vs. saline-treated diabetic group; **P<0.05 vs. saline-treated non-diabetic group.
rats (Suzuki et al., 2003). In agreement with previous studies, a rat model of diabetic osteopenia was successfully established by injection of streptozotocin in the present study, which was characterized by elevated serum glucose level, decreased bone mineral density, decreased bone biomechanical quality and impaired micro-architecture of the femurs. In addition, both inhibited bone formation and bone resorption were also observed in the present study, suggesting a decreased bone remodeling in diabetic osteopenia in the current study.

Quercetin was capable of lowering the serum glucose level in streptozotocin induced diabetes in rats in the present study. It has
been shown that quercetin exerts protective effects against β-cell damage in diabetes by decreasing oxidative stress and preservation of β-cell integrity (Coskun et al., 2005). In their report, streptozotocin injection significantly increased lipid peroxidation in pancreatic tissue and serum nitric oxide concentration, as well as decreased the antioxidant enzyme activities. In addition, damaged β-cells were extensively degranulated, degenerated or necrosed in the streptozotocin treated rats, which lead to decreased insulin secretion and increased serum glucose concentration. Administration of quercetin partially prevents degranulation and degeneration of β-cell, and significantly lowered the serum glucose levels. In addition, the lipid peroxidation in pancreatic tissue, serum nitric oxide production was significantly lowered, and the activities of serum antioxidants were significantly increased by quercetin, indicating that the protective effect of quercetin on β-cell is attributable to its antioxidant nature. In the present study, the elevated serum glucose induced by streptozotocin was significantly lowered by quercetin, suggesting a beneficial effect of quercetin on pancreatic tissue in diabetic rats. The hyperglycemia has been shown to lead to the incidence of osteopenia in diabetic rats. Therefore, the beneficial effect of quercetin on pancreatic tissue, and its capability of lowering serum glucose level might be, at least in part, responsible for the protective effect of quercetin on diabetic osteopenia in the present study.

Hyperglycemia is able to trigger increased oxidative stress, which has been considered to be involved in the pathogenesis of diabetic bone disorders (Hamada et al., 2009; Wauquier et al., 2009). Under diabetic conditions, the oxidative stress significantly increased, which could induce cellular dysfunctions in a variety of cell types including bone cells (Bai et al., 2004; Hamada et al., 2009; King and Loeken, 2004). In the present study, increased oxidative stress was found in diabetic rats, which was evidenced by increased urinary 8-OHdG level (a sensitive indicator of oxidative DNA damage), decreased serum antioxidant capability and decreased activity of serum antioxidants. Quercetin, a potent antioxidant, was found to be able to reduce the extent of oxidative stress in diabetic rats in the present study. After treatment with quercetin (30 mg/kg and 50 mg/kg), the urinary 8-OHdG level in diabetic rats significantly decreased, and the activities of serum antioxidants significantly increased, suggesting that administration of quercetin was capable of lowering the extent of oxidative stress in diabetic rats. The beneficial effect of quercetin on oxidative stress might be achieved through direct or indirect ways. On one hand, the potent antioxidant capability of quercetin makes it possible to lower the systematic oxidative stress directly under diabetic conditions. On the other hand, quercetin could lower the serum glucose, which has been shown to trigger increased oxidative stress in diabetic rats (King and Loeken, 2004). Therefore, the effect of quercetin on oxidative stress might also be realized indirectly through lowering the concentration of serum glucose, which subsequently triggered a lower extent of oxidative stress in diabetic rats. Although we identified the beneficial effect of quercetin on oxidative stress, the mechanisms underlying the effect of quercetin on oxidative stress under diabetic condition still need to be identified in future studies.

Quercetin (30 mg/kg and 50 mg/kg) was capable of partially reversing the diabetic osteopenia in the present study. The decreased bone mass induced by diabetes significantly increased after oral administration of quercetin (30 mg/kg and 50 mg/kg) for 8 weeks. In addition, both bone formation and bone resorption were inhibited in diabetic rats, which were evidenced by serum/urinary markers and histomorphometric analysis. Further studies showed that quercetin was capable of partially reversing the inhibited bone formation and bone resorption, and consequently improved the loss of bone mass in diabetic rats. In the present study, it has been shown that quercetin could lower the serum glucose and oxidative stress, which has been recognized as important contributors to the development of diabetic osteopenia. Therefore, the beneficial effect of quercetin on diabetic osteopenia might be attributable to its ability in lowering the concentration of serum glucose and oxidative stress. In addition, the effect of quercetin on diabetic osteopenia might also be related with its action on cells related to bone remodeling. It has been reported that quercetin was capable of increasing osteogenic activity in MG-63 osteoblasts (Prouillet et al., 2004), as well as influencing the osteoclastic resorption in osteoclasts in vitro (Wattel et al., 2004), indicating a possible effect of quercetin on the functions of cells related to bone remodeling under diabetic condition. It has been shown that the cellular behaviors of bone marrow stromal cells, osteoblast, and osteoclast under diabetic conditions are significantly different from those under normal condition (Guan et al., 2009; Kim et al., 2009; Verhaeghe et al., 1997; Wittrant et al., 2008; Zhen et al., 2010). The effect of quercetin on these cells under diabetic conditions might be one of the contributing factors to its beneficial effect on diabetic osteopenia, and this interesting topic still needs to be identified in future studies.

The biomechanical quality of bone is an important factor reflecting bone fragility and fracture risk, which is determined by bone size, shape, and material property (Turner and Burr, 1993). In both previous and present studies, hyperglycemia induced by streptozotocin was capable of impairing the mechanical integrity of bone and decreasing the ability to resist fracture in bone, which is evidenced by decreased maximum load, stiffness, Young modulus and energy absorption levels. In previous studies, the micro-structure of trabecular bone and cortical bone, which was examined by Micro-CT, has been found to be partially destroyed by hyperglycemia (Thrailkill et al., 2005). In the present study, alterations in both trabecular bone and cortical bone (CaR and CTh) was revealed in diabetic rats by Micro-CT analysis, which might contribute to the deteriorative biomechanical performance of femur under diabetic condition. Further investigations showed that oral administration of quercetin (30 mg/kg and 50 mg/kg) significantly improved the biomechanical quality of bone in diabetic rats, which occurred coincidentally with an improvement in the architectural parameters in both trabecular and cortical bone in quercetin-treated diabetic rats. Therefore, the efficiency of quercetin on the deteriorative biomechanical performance of femur in diabetic rats might be attributable to the improvement in the micro-structure of trabecular and cortical bone in quercetin-treated diabetic rats.

In conclusion, the present study showed that oral administration of quercetin (30 mg/kg and 50 mg/kg) was capable of partially reversing osteopenia in diabetic rats. The beneficial effect of quercetin on diabetic osteopenia might be related to its antioxidant property and its ability on lowering serum glucose. The findings in the present study suggest the possibility of developing quercetin as a drug or an ingredient in diet for the treatment of diabetic osteopenia.

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References


