DPP IV inhibitor treatment attenuates bone loss and improves mechanical bone strength in male diabetic rats

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Glorie L, Behets GJ, Baerts L, De Meester I, D’Haese PC, Verhulst A. DPP IV inhibitor treatment attenuates bone loss and improves mechanical bone strength in male diabetic rats. Am J Physiol Endocrinol Metab 307: E447–E455, 2014. First published July 22, 2014; doi:10.1152/ajpendo.00217.2014.—Dipeptidyl peptidase IV (DPP IV) modulates protein activity by removing dipeptides. DPP IV inhibitors are currently used to improve glucose tolerance in type 2 diabetes patients. DPP IV substrates not only increase insulin secretion but also affect bone metabolism. In this study, the effect of DPP IV inhibitor sitagliptin on bone was evaluated in normal and streptozotocin-induced diabetic rats. This study included 64 male Wistar rats divided into four groups (n = 16): two diabetic and two control groups. One diabetic and one control group received sitagliptin through drinking water. Tibiae were scanned every 3 wk using an in vivo μCT scanner. After 6 and 12 wk, rats were euthanized for histomorphometric analysis of bone parameters. The mechanical resistance of femora to fracture was assessed using a three-point bending test, and serum levels of bone metabolic markers were measured. Efficient DPP IV inhibition was achieved in sitagliptin-treated groups. Trabecular bone loss, the decrease in trabecular number, and the increase in trabecular spacing was attenuated through sitagliptin treatment in diabetic rats, as shown by in vivo μCT. Bone histomorphometry was in line with these results. μCT analysis furthermore showed that sitagliptin prevented cortical bone growth stagnation in diabetic rats, resulting in stronger femora during three-point bending. Finally, the serum levels of the resorption marker CTX-I were significantly lower in sitagliptin-treated diabetic animals compared with untreated diabetic animals. In conclusion, sitagliptin treatment attenuates bone loss and increases bone strength in diabetic rats probably through the reduction of bone resorption and independent of glycemic management.

dipeptidyl peptidase IV; dipeptidyl peptidase IV inhibition; incretins; sitagliptin; diabetes

DIABETES MELLITUS IS A GROUP OF METABOLIC DISEASES characterized by high levels of blood glucose (hyperglycemia) in a fasting state caused by either an insufficient production of insulin (type 1 diabetes) or a progressively reduced insulin receptor sensitivity (type 2 diabetes). This metabolic disorder is accompanied with the development of substantial bone pathology; the incidence of fractures is increased in type 1 as well as in type 2 diabetic patients, indicating a diminished bone quality in the entire diabetic population (10, 18). In the general population, bone mineral density (BMD) is inversely correlated with fracture risk. This association is also seen in type 1 diabetic patients, which are osteopenic as reflected by a low BMD; however, no such association exists in type 2 diabetic patients (1, 46), who have either a normal or slightly increased BMD. Glycemic control is crucial for bone health in diabetics, as a chronic hyperglycemic state impairs osteoblast function, which is considered the onset of diabetic osteopenia (18). A hyperglycemic state also diminishes the response of osteoblasts and osteoclasts to vitamin D, which is important because the diabetic population tends to have lower levels of vitamin D compared with a nondiabetic population (57). Moreover, diabetic bone is susceptible to nonenzymatic glycation, by which the collagen contains increased amounts of advanced glycation end products (AGEs), which in turn can impair bone mineralization, resulting in the diminished bone quality. So although in diabetes BMD may increase, it goes along with loss of bone strength (18).

Dipeptidyl peptidase IV (DPP IV) is a ubiquitously expressed ectopeptidase that exists in a soluble and a membrane-bound form. It can be found on the surface of mostly epithelial and endothelial cells but also activated immune cells. Highest expression levels in humans have been reported to occur in the intestine, the bone marrow, and the kidney (28). DPP IV modulates the activity of the incretins glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) but also the activity of a series of other substrates (38) that have important functions in metabolism, inflammation, cell migration and differentiation, brain and nerve cells, and the bone (8, 41). DPP IV inhibitors were approved by the FDA in 2006 as a treatment to improve the glucose tolerance of type 2 diabetic patients, as they increase the half-life of the incretins GIP and GLP-1, which bind receptors on the β-pancreatic cells, thereby promoting the secretion of insulin.

The effect of increasing the half-life of these substrates through DPP IV inhibition has been investigated extensively over the years (27, 43). DPP IV inhibitors were shown to exert positive effects on the heart and vessels (35, 37, 49) and the lungs and the kidney, as evidenced in animal models of myocardial infarction (3, 59), lung transplantation (23, 58), and acute (16) and chronic (22) renal failure, respectively. A number of DPP IV substrates also influence the bone, since they have receptors on active bone cells. These include gut hormones (GIP, GLP-1, and GLP-2) and peptides influencing satiety and hunger [neuropeptide Y (NPY) and peptide YY (PYY)] but also stromal cell-derived factor-1α, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), substance P (SP), and insulin-like growth factor 1 (IGF-1). Through various experiments using DPP IV substrate and receptor knockouts and the exogenous administration of receptor agonists in experimental as well as clinical studies, many DPP IV substrates have been shown to positively affect bone metabolism, suggesting a positive effect of DPP IV...
inhibition on the bone (2, 4, 15, 17, 24, 29, 31, 32, 40, 51–54, 56). Furthermore, DPP IV activity is correlated with blood glucose levels as well as AGEs (48), and DPP IV inhibitors have been shown to directly influence the AGE-receptor for advanced glycation end products axis (37). Literature data on the in vivo effects of DPP IV inhibitors on bone metabolism are scarce, notwithstanding the high number of preclinical and clinical studies with these compounds. In a clinical meta-analysis, DPP IV inhibition has been associated with a reduced risk of bone fractures (39), but the same association could not be made for the use of GLP-1 receptor agonists (33). The observed effect of DPP IV inhibitors on the bone metabolism, independent of glucose levels, has not been confirmed previously in experimental studies (14, 26, 45).

In rats, injection of nicotinamide (NAD) and streptozotocin (STZ) results in a partial destruction of pancreatic β-cells, leading to an insulinopenia and hyperglycemia (34). The STZ-treated rat was found previously to display a reduced bone turnover, hypercalcemia, hyperphosphatemia, and decreased serum parathyroid hormone (PTH) levels (19, 47). In Wistar and other rat strains, NAD-STZ treatment was also found to induce a loss of mechanical resistance of the bone (13, 42). In this study, the effect of oral administration of sitagliptin on the architectural properties of mineralized bone as well as bone strength is assessed and characterized in the NAD-STZ-induced diabetic as well as in the healthy Wistar rat.

MATERIALS AND METHODS

Experimental Setup

All procedures were carried out in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (7th ed., 1996) following approval by the University of Antwerp Ethics Committee. In this experiment, 64 male Wistar rats (10 wk of age, 300–325 g; Iffa Credo, Brussels, Belgium) were randomly assigned to four experimental groups, two control groups (10 wk of age, 300–325 g; Iffa Credo, Brussels, Belgium) and two diabetic groups, each one treated orally with 2 g/l sitagliptin (10 wk of age, 300–325 g; Iffa Credo, Brussels, Belgium). The remaining animals were euthanized 6 wk after induction of diabetes by exsanguination through the abdominal aorta after pentobarbital sodium anaesthesia [Nembutal(R); Ceva Santé Animale, Brussels, Belgium]; the remaining animals were euthanized after 12 wk.

Biochemical Analyses

DPP IV enzymatic activity was assayed in rat serum, using glycyl-prolyl-4-methoxy-β-naphthylamide as a fluorogenic substrate. The in vitro inhibition of the DPP IV activity is an underestimation of the in vivo inhibition due to dissociation of the reversible inhibitor-DPP IV complex during sample dilution in the assay. The percentage of in vivo DPP IV activity was estimated according to the method described by Matheussen et al. (36).

Serum levels of osteocalcin and PTH in diabetic animals were determined using ELISA kits from Immutopics (San Clemente CA). The levels of COOH-terminal peptide of collagen I (sCTX-I) and pentosidine (as a marker of AGEs) were determined in the serum of diabetic animals with ELISA kits from USCNK (Uscn Life Science, Hubei, China).

Creatinine was determined in serum and urine using a modified method based on the picric acid method first described by Jaffé (21). Serum and urinary phosphate were determined using the Phosphate FS* kit by DiaSys (Diagnostic Systems, Holzheim, Germany). Serum and urinary calcium were determined by flame atomic absorption spectrometry, using a Perkin-Elmer AutoAnalyser 800.

Table 1. Weight, glycemia, and DPP IV activity of animals at the start and the end of the experiment

<table>
<thead>
<tr>
<th></th>
<th>CV</th>
<th>C/SG</th>
<th>DM/V</th>
<th>DM/SG</th>
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<tr>
<td>Initial weight, g</td>
<td>330 ± 18</td>
<td>317 ± 24</td>
<td>320 ± 10</td>
<td>321 ± 14</td>
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<tr>
<td>Final weight, g</td>
<td>412 ± 23</td>
<td>378 ± 26</td>
<td>312 ± 36†</td>
<td>324 ± 73†</td>
</tr>
<tr>
<td>Femoral length, mm</td>
<td>38.13 ± 0.83</td>
<td>38.13 ± 1.13</td>
<td>36.88 ± 0.35†</td>
<td>36.71 ± 1.25†</td>
</tr>
<tr>
<td>Week 6</td>
<td>38.57 ± 1.13</td>
<td>38.5 ± 1.38</td>
<td>36.86 ± 0.69†</td>
<td>37.14 ± 1.95</td>
</tr>
<tr>
<td>Week 12</td>
<td>125 ± 6</td>
<td>120 ± 7</td>
<td>121 ± 7</td>
<td>121 ± 7</td>
</tr>
<tr>
<td>Initial glycemia, mg/dl</td>
<td>136 ± 15</td>
<td>120 ± 6†</td>
<td>392 ± 137†</td>
<td>390 ± 171†</td>
</tr>
<tr>
<td>Final glycemia, mg/dl</td>
<td>11.5 ± 9.7</td>
<td>9.3 ± 6.1</td>
<td>28.8 ± 11.9†</td>
<td>19.1 ± 12.6</td>
</tr>
<tr>
<td>Food consumption, g</td>
<td>14.6 ± 9.0</td>
<td>10.0 ± 10.4</td>
<td>32.0 ± 17.4†</td>
<td>14.5 ± 11.9*</td>
</tr>
<tr>
<td>Week 6</td>
<td>18.0 ± 6.1</td>
<td>9.1 ± 5.5†</td>
<td>109.5 ± 52.8†</td>
<td>60.3 ± 45.9**</td>
</tr>
<tr>
<td>Week 12</td>
<td>21.7 ± 10.8</td>
<td>9.7 ± 8.6†</td>
<td>127.4 ± 93.3†</td>
<td>38.8 ± 40.2*</td>
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<tr>
<td>Urine volume, ml</td>
<td>16.5 ± 6.1</td>
<td>7.5 ± 4.2†</td>
<td>102.3 ± 57.3†</td>
<td>66.9 ± 52.6†</td>
</tr>
<tr>
<td>Week 6</td>
<td>14.3 ± 7.6</td>
<td>6.7 ± 3.5†</td>
<td>91.7 ± 53.1†</td>
<td>51.0 ± 39.5†</td>
</tr>
<tr>
<td>Creatinine clearance, ml-min⁻¹·100 g⁻¹</td>
<td>0.33 ± 0.04</td>
<td>0.26 ± 0.09</td>
<td>0.36 ± 0.11</td>
<td>0.33 ± 0.13</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.35 ± 0.08</td>
<td>0.32 ± 0.09</td>
<td>0.39 ± 0.10</td>
<td>0.38 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 16. DPP IV, dipeptidyl peptidase IV; CV, untreated control animals; C/SG, controls treated with sitagliptin; DM/V, untreated diabetic animals; DM/SG, diabetic animals treated with sitagliptin. *P < 0.05 compared with DM/V; †P < 0.05 compared with CV.
Microcomputed Tomography

The proximal ends of the right tibiae of all animals were evaluated by in vivo microcomputed tomography (μCT) every 3 wk using a SkyScan 1076 μCT scanner after intravenous pentobarbital sodium anesthesia. A resolution of 34.75 μm was chosen to be able to scan all animals in the experimental groups, using a minimum of anesthesia and avoiding artefacts due to animal movement, while maintaining a minimum object to pixel ratio for accurate measurements (6, 11). Resulting images were reconstructed using the SkyScan software NRecon, and a total bone length of 3.5 mm in the secondary spongiosa at a distance of 1.75 mm from the growth plate was analyzed using CtAnalyser (SkyScan software). Trabecular and cortical bone were quantified separately in the metaphyseal region. Results are given as percentages relative to the baseline value of each animal.

Bone Histomorphometry

The right tibiae were dehydrated in increasing ethanol concentrations and impregnated for 1 wk with methyl methacrylate monomer, after which polymerization was allowed to proceed for 48 h under an N₂ atmosphere. Five-micrometer-thick Goldner-stained sections were used to perform histomorphometric analysis. The sections were analyzed using Axiovision (Release 4.5; Carl-Zeiss), a semiautomatic image analysis system. Bone area, osteoid area, osteoid perimeter, eroded perimeter, and quiescent perimeter were measured in consecutive fields with a total surface of 1.5–2 mm² at a distance of 0.66 mm from the growth plate in the secondary spongiosa by manually tracing the mineralized and osteoid area and marking erosion, osteoblasts, and osteoclasts on the computer screen, after which the system calculated the areas and perimeters. Cuboidal cells covering the osteoid seams were considered active osteoblasts, whereas large multinucleated cells laying within resorption lacunae were considered active osteoclasts. Secondary parameters were calculated according to standardized procedures (12).

Three-Point Bending

Strength of harvested femora was measured using a three-point bending setup with a 100-N load cell and a pressure transducer. In this setup, the distance between both resting points was 35 mm, and the bending point was positioned in the middle of the diaphysis. Displacement, coordinated at a speed of 5 μm/s in the antero-posterior axis, and applied force were measured and communicated to Mathlab...

Fig. 1. Ratio of trabecular bone volume over tissue volume (BV/TV; A), trabecular spacing (B), trabecular number (C), and cortical bone volume (D) in the metaphysis as assessed by microcomputed tomography (μCT) analysis. Change relative to individual baseline value (%). *P < 0.05 in diabetic animals treated with vehicle (DM/V) compared with controls treated with vehicle (C/V). DM/V, and DM/SG. E: 3-dimensional images of the metaphysis reconstructed by CtAnalyser [from left to right: C/V, controls treated with sitagliptin (C/SG), DM/V, and DM/SG].
software. Stiffness, ultimate load, ultimate displacement, and energy to failure were determined from the force-displacement curves registered by Mathlab. After the femoral bones were broken, the geometrical properties of the cortical surface at the diaphysis were measured by μCT proximal from the breaking point, which was at a similar position in all subjects, to derive a stress-strain curve to determine ultimate stress, ultimate strain, Young’s modulus, and toughness (44, 50).

Statistical Analysis

Results are given as means ± SD. Bone histomorphometric data are presented as individual data and median. Statistics were performed with IBM SPSS Statistics 20. Comparisons between study groups were assessed using the Kruskal-Wallis H-test, followed by a Mann-Whitney U-test in combination with the Bonferroni correction when more than two groups were compared. Values of $P < 0.05$ were considered significant.

Table 2. Mechanical properties of the right femoral bone, as measured with 3-point bending and calculated using MathLab

<table>
<thead>
<tr>
<th></th>
<th>C/V</th>
<th>C/SG</th>
<th>DM/V</th>
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<tr>
<td>Stiffness, N/mm</td>
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<tr>
<td>Week 6</td>
<td>154.8 ± 19.0</td>
<td>148.4 ± 17.0</td>
<td>135.8 ± 11.3</td>
<td>150.0 ± 15.2</td>
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<td>Week 12</td>
<td>173.4 ± 16.46</td>
<td>175.8 ± 15.3</td>
<td>140.4 ± 26.3</td>
<td>165.0 ± 25.9</td>
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<tr>
<td>Ultimate load, N</td>
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<tr>
<td>Week 6</td>
<td>87.76 ± 9.50</td>
<td>82.78 ± 8.01</td>
<td>74.86 ± 5.00†</td>
<td>84.52 ± 11.78</td>
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<tr>
<td>Week 12</td>
<td>88.03 ± 11.96</td>
<td>90.96 ± 9.77</td>
<td>66.60 ± 11.06†</td>
<td>81.96 ± 16.12*</td>
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<tr>
<td>Ultimate load/cortical transversal surface, N/mm²</td>
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<tr>
<td>Week 6</td>
<td>12.31 ± 0.97</td>
<td>12.02 ± 1.38</td>
<td>11.59 ± 0.63</td>
<td>12.66 ± 1.22</td>
</tr>
<tr>
<td>Week 12</td>
<td>11.74 ± 1.36</td>
<td>12.58 ± 1.13</td>
<td>10.65 ± 1.26†</td>
<td>12.04 ± 1.29*</td>
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<tr>
<td>Ultimate displacement, mm</td>
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<tr>
<td>Week 6</td>
<td>0.65 ± 0.07</td>
<td>0.75 ± 0.27</td>
<td>0.63 ± 0.09</td>
<td>0.66 ± 0.13</td>
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<tr>
<td>Week 12</td>
<td>0.57 ± 0.12</td>
<td>0.55 ± 0.03</td>
<td>0.56 ± 0.11</td>
<td>0.55 ± 0.13</td>
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<tr>
<td>Energy to failure, mJ</td>
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<td>Week 6</td>
<td>2.86 ± 0.73</td>
<td>2.79 ± 1.10</td>
<td>2.36 ± 0.84</td>
<td>2.93 ± 1.33</td>
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<td>Week 12</td>
<td>2.25 ± 1.32</td>
<td>2.00 ± 0.29</td>
<td>1.60 ± 0.45</td>
<td>2.09 ± 1.14</td>
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<td>Young’s modulus, MPa/°</td>
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<td>Week 6</td>
<td>402.3 ± 44.6</td>
<td>391.0 ± 53.9</td>
<td>400.2 ± 51.3</td>
<td>376.0 ± 100.2</td>
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<td>Week 12</td>
<td>411.9 ± 55.7</td>
<td>424.9 ± 36.0</td>
<td>401.1 ± 63.8</td>
<td>417.4 ± 39.7</td>
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<tr>
<td>Ultimate stress, MPa</td>
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<td>Week 6</td>
<td>279.0 ± 21.3</td>
<td>277.2 ± 25.4</td>
<td>262.3 ± 29.2</td>
<td>261.4 ± 66.0</td>
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<tr>
<td>Week 12</td>
<td>512.1 ± 38.2</td>
<td>529.6 ± 23.6</td>
<td>481.4 ± 74.1</td>
<td>513.9 ± 34.3</td>
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<td>Ultimate strain, %</td>
<td></td>
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<tr>
<td>Week 6</td>
<td>8.0 ± 0.9</td>
<td>9.4 ± 4.2</td>
<td>7.5 ± 1.2</td>
<td>8.4 ± 1.9</td>
</tr>
<tr>
<td>Week 12</td>
<td>7.1 ± 1.7</td>
<td>6.9 ± 0.6</td>
<td>6.7 ± 1.3</td>
<td>6.9 ± 1.7</td>
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<tr>
<td>Toughness, J/mm³</td>
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<tr>
<td>Week 6</td>
<td>9.02 ± 2.03</td>
<td>9.30 ± 3.43</td>
<td>8.21 ± 2.73</td>
<td>8.95 ± 4.49</td>
</tr>
<tr>
<td>Week 12</td>
<td>6.69 ± 3.58</td>
<td>6.01 ± 0.45</td>
<td>5.54 ± 1.62</td>
<td>6.35 ± 2.98</td>
</tr>
</tbody>
</table>

Values are means ± SD *$P < 0.05$ compared with DM/V; †$P < 0.05$ compared with C/V.

RESULTS

Evaluation of Body Weight, Growth, Glucose Levels, and Metabolic Cage Data

Results evaluating the body weight, growth, glucose levels, and metabolic cage data are presented in table 1. No significant differences in the initial weight of the control vs. the diabetic group were observed. As expected, at the end of the experiment, the diabetic groups displayed a significantly decreased body weight compared with control groups. Femoral length was found to be significantly lower in diabetic animals compared with nondiabetic animals at weeks 6 and 12, except for the sitagliptin-treated diabetic animals at week 12, of which the femoral length did not differ from the control groups. In the diabetic animals, a significantly increased glycemia was observed compared with controls. Throughout the experiment,
sitagliptin treatment did not have any effect on glucose levels in the diabetic group. STZ-treated animals had a significantly increased food consumption, drinking volume, and urine volume compared with control animals. Treatment with sitagliptin lowered each one of these parameters, with a significant reduction in food consumption at week 12 and drinking volume at weeks 6 and 12. A similar but nonsignificant trend was observed for the urine volume. Drinking and urine volume were also significantly lowered by sitagliptin treatment in controls. Renal function as assessed by creatinine clearance did not differ between groups.

**DPP IV Activity**

Treatment with sitagliptin in the drinking water resulted in an average inhibition of serum DPP IV activity of 86.6 ± 3.5 and 86.2 ± 3.3% in diabetic and control animals, respectively. This efficient inhibition was seen in diabetic as well as control animals despite variable dosage due to increased water consumption in the diabetic groups (Table 1).

**μCT Analysis**

**Trabecular bone.** μCT analysis of the trabecular bone structure in the metaphysis of the tibiae revealed distinct structural differences between diabetic animals and controls, as can be seen in Fig. 1E. The ratio of the trabecular bone volume over the total cancellous tissue volume (BV/TV) was found to be significantly lower in diabetic animals at weeks 6, 9, and 12 (Fig. 1A). The loss of trabecular bone in diabetic animals was completely attenuated by sitagliptin treatment. BV/TV of these animals was preserved compared with untreated diabetic animals at weeks 9 and 12 and at no time point differed significantly from the nondiabetic controls.

No significant difference in trabecular thickness was observed between animal groups. Trabecular spacing increased in...
all groups over time, which was significantly more pronounced in diabetic animals, as evidenced by a threefold increase compared with an only twofold increase in control animals. This difference became significant at weeks 9 and 12. In sitagliptin-treated diabetic animals, the effect of diabetes on trabecular spacing was completely abolished and at no time point differed significantly from the control animals. Compared with the untreated diabetic animals, the trabecular spacing was significantly lower at week 12 (Fig. 1B).

The number of trabeculae decreased steadily in all groups. In untreated diabetic animals, the trabecular number decreased by >60% compared with almost 40% in controls, resulting in a significant difference between both groups from week 3 until the end of the experiment (Fig. 1C). The trabecular number of diabetic animals treated with sitagliptin at no time point differed from the untreated nondiabetic controls and was significantly higher compared with untreated diabetic animals at week 9.

Cortical bone. The cortical bone volume of the tibiae as measured with μCT in the metaphysis increased steadily with time in the control animals, whereas the cortical bone growth was significantly lower in diabetic animals and stagnated, resulting in a significant difference vs. control animals from week 3 onward. The cortical bone volume of the sitagliptin-treated diabetic animals stagnated by week 6 at a higher level compared with the untreated diabetic animals and was at no point significantly different from the control animals (Fig. 1D).

Three-Point Bending

In diabetic animals, a significantly lower ultimate load and thus mechanical strength were observed compared with controls in week 6 as well as in week 12. Sitagliptin treatment prevented the loss of strength in diabetic bone, as the ultimate load measured was significantly higher than that in untreated diabetic animals at week 12. When the load over the transversal cortical bone surface determined by μCT was normalized, this difference remained significant (Fig. 2). Neither diabetes nor sitagliptin had any significant effect on bone stiffness, ultimate displacement, energy to failure, Young’s modulus, ultimate strain, ultimate stress, or toughness (Table 2).

Histomorphometric Analysis of Bone Area and Trabecular Spacing/Number

The results of the histomorphometric analysis of bone area and trabecular spacing/number (Fig. 3), which are also reflected by representative histological sections of Fig. 4, lead us to suggest a confirmation of the significant effect of sitagliptin on diabetic bone pathology observed by μCT and three-point bending. The absence of significant differences, however, can at least in part be ascribed to the high biological variability inherent to the bone histomorphometric analysis.

Evaluation of Osteoblast and Osteoclast Activity

To provide further support for the observed differences between sitagliptin-treated and untreated diabetic animals, bone metabolic biomarkers were determined. Serum osteocalcin levels showed a decrease in all diabetic animals in weeks 6 and 12 compared with baseline (Fig. 5, left). In line herewith, the amount of osteoid, as well as the number of active osteoblasts observed during histomorphometric analysis, was relatively low and similar in all groups (data not shown). Serum CTX-I level, a measurement of osteoclast activity, increased drastically in all diabetic animals at week 6 compared with baseline. Interestingly, at week 12 CTX-I levels were significantly lower in sitagliptin-treated diabetic animals compared with untreated diabetic animals (Fig. 5, middle). This is in line with the histomorphometric analysis of the number of osteoclasts at this time point, as shown in Fig. 5, right. No differences were observed when the PTH and pentosidine levels of sitagliptin-treated and untreated diabetic animals were compared (data not shown).

Urinary Calcium, Phosphate, and Creatinine

Induction of diabetes resulted in a significantly increased excretion of calcium and a nonsignificant increase in phosphate in the urine compared with control animals (Fig. 6). Sitagliptin treatment did not affect the urinary excretion of calcium and phosphate. Concentrations of calcium and phosphate in the serum were similar in all animals (data not shown). NAD-STZ treatment did not influence serum creatinine levels, nor did it affect the creatinine clearance (Table 1).

DISCUSSION

This study investigates the effect of an efficient inhibition of DPP IV activity on the development of diabetic bone pathology and, for the first time, clearly shows a beneficial effect of DPP IV inhibitor treatment on bone mass as well as bone strength in diabetic animals independent of their blood glucose levels. In this study, we did not measure the insulinemia of the animals, nor did we include additional insulin-treated groups, which can be considered a limitation of the study. Hyperglycemia, which was previously shown to result in an impairment of bone cellular activity (5, 9, 18), remained unaffected through sitagliptin treatment in diabetic groups, indicating that differences in osteoclastic activity observed in our study cannot be ex-
plained by differences in glycemia. In an experimental study published in 2010, a positive effect of sitagliptin was observed on vertebral bone volume and trabecular architecture in high-fat diet-fed female mice, but this effect was associated with a significant reduction of the blood glucose levels (26), which is not surprising as in contrast to our study they used a type 2 diabetic model with only moderate hyperglycemia. Another recent experimental study concluded that experimental inhibitor MK-0626 did not affect bone quality in diabetic MKR mice (14), which might be due to the effect of the mutation in the IGF-I receptor in this model, which was shown to reduce proliferation of bone cells as well as osteoblast activity (17) and also indirectly influence other incretins (4).

As shown in our study, treatment with sitagliptin results in a significant attenuation of trabecular bone loss and a marked improvement of trabecular bone structure, which can be attributed to the conservation of trabecular number and trabecular spacing, without trabecular thickness being affected. Sitagliptin treatment in diabetic animals also resulted in a continued cortical bone growth (bone volume) compared with untreated diabetic animals. A similar trend, but one limited to the trabecular bone, was observed in the sitagliptin-treated control animals. Furthermore, a significant increase was observed in the ultimate load to fracture the femora of sitagliptin-treated vs. untreated diabetic animals. Bone histomorphometry results from, to a certain extent, mirrored measurements made by μCT, which has also been reported by others (7). Furthermore, serum CTX-I concentrations were significantly lower in sitagliptin-treated animals, whereas serum osteocalcin levels remained unaffected, suggesting that the sitagliptin-induced effect on the bone was not achieved by influencing bone-forming cells but by reducing the number of bone-resorbing cells. It would be interesting for further research to investigate the effect of DPP IV inhibition on osteoclasts in vitro.

The sitagliptin treatment had no effect on measured serum PTH levels or on serum pentosidine levels, suggesting that the effect of sitagliptin on bone cannot be attributed to changes in circulating AGEs or to interaction at the level of the parathyroid gland.

The bone phenotype of the controls seems to remain unaffected by sitagliptin treatment. Whether this is due to inflammatory processes, hyperglycemia, variations of circulating DPP IV substrate concentrations, or variations of substrate receptor expression in diabetic animals compared with healthy animals remains to be elucidated. In this regard, it is worth mentioning that serum levels of GIP, GLP-1, and other DPP IV substrates as well as the expression of their receptors were found to be altered through hyperglycemia in experimental as well as clinical studies (20, 25, 30, 55).

Further research is needed to assign the observed improvement of bone quality to the activity of DPP IV substrates. The decreased bone resorption in sitagliptin-treated diabetic animals might be the result of a prolonged half-life of DPP IV substrates, of which the beneficial effects on bone metabolism have been shown previously. In previous experiments, the
effect of GLP-1 (40), GLP-2 (2), and GIP (54) on bone resorption has been clearly shown. Nevertheless, the effects of neuropeptides and immunomodulators like VIP (29, 31), PACAP (52), NPY (32), PYY (53), SP (29), and IGF-1 (56), which also influence the activity of osteoclasts directly, should also be taken into account. Besides having a direct effect on the activity of osteoclasts, the latter substrates may also exert an effect on bone metabolism through feeding and energy regulation (24, 51). Thus the observed effect on bone-resorbing cells is most probably a combined effect of different DPP IV substrates on these cells. The affinity of DPP IV for these substrates, but also the organs in which they are synthesized as well as their exposure to DPP IV in their journey toward the bone and the affinity of these substrates to the present receptors, has to be taken into account in the evaluation of the effect of these substrates.

This study presents the clear observation that sitagliptin treatment results in increased cortical and trabecular bone volume as well as an increased bone strength in diabetic animals compared with untreated diabetic animals. The results of this study strongly suggest a protective effect of DPP IV inhibition on the bone in diabetic animals, which might be the reason for a reduced fracture risk in the diabetic population treated with DPP IV inhibitors (39). Because diabetic patients have an increased risk of fractures combined with an increased tendency to fall due to peripheral neuropathy, diabetic retinopathy, and abnormal fluctuations in glycemia and blood pressure, resulting in light-headedness, these results may prove to be an additional clinical potential of DPP IV inhibitors in maintaining the strength of bone compromised by impaired glucose tolerance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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