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# Type 2 Diabetes and Metformin Influence on Fracture Healing in an Experimental Rat Model

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ABSTRACT

Persons with diabetes have a greater incidence of fractures compared with persons without diabetes. However, very little published information is available concerning the deleterious effect of late-stage diabetes on osseous structure and bone healing. The purpose of the present study was to evaluate the role of diabetes on fracture healing in a rat femur repair model. Thirty-six lean and diabetic Zucker rats were subdivided into 3 groups: (1) 12 lean rats as the control group; (2) 12 diabetic rats without blood glucose control (DM group); and (3) 12 diabetic rats treated with 300 mg/kg metformin to reduce the blood glucose levels (DM + Met group). Radiographs were taken every week to determine the incidence of bone repair and delayed union. All the rats were killed at 6 weeks after surgery. In both the sham-operated and the fractured and repaired femurs, significant decreases in the fracture-load/weight and marginal decreases in the fracture-load between the lean and DM groups were found. Metformin treatment significantly reduced the blood glucose and body weight 12 days postoperatively. Furthermore, a decrease in the fracture-load and fracture-load/weight in the repaired femurs was found in the DM + Met group. Diabetes impairs bone fracture healing. Metformin treatment reduces the blood glucose and body weight but had an adverse effect on fracture repair in diabetic rats. Further investigations are needed to reveal the mechanisms responsible for the effects of type 2 diabetes mellitus on bone and bone quality and the effect of medications such as metformin might have in diabetic bone in the presence of neuropathy and vascular disease.



People with diabetes have a greater incidence of fractures compared with persons without diabetes (1–3). Diabetes is also more prevalent in the elderly and, therefore, frequently coexists with osteoporosis (3). Furthermore, a global increase has occurred in the prevalence of obesity, with obesity-related diabetes currently affecting >366 million adults worldwide, with projections that this will reach 552 million by 2030 (4). The evidence that fracture risk is increased in those with type 2 diabetes mellitus (T2DM), despite normal bone mineral density, has led to the hypothesis that diabetes-associated alterations occur in skeletal properties (2,5). These alterations, which potentially include abnormalities in material and microarchitectural properties, might contribute to the increased fracture risk with T2DM.

High glucose levels in T2DM lead to the accumulation of advanced glycosylation end products (AGEs) in the organic bone matrix by a process known as nonenzymatic glycation (the Maillard reaction) (6–8). Increased AGEs can weaken bone by decreasing bone formation. Evidence has suggested that AGEs interfere with normal osteoblast development (9), function (10), and attachment to the collagen matrix (11). Moreover, low bone formation also works in parallel to further increase AGEs, such as with high bisphosphonate dosages (12). Recent studies have suggested that in T2DM, the trabecular bone mass and structure are intact and perhaps even enhanced, but the cortical compartment is preferentially compromised (13,14).

Most studies have documented the effects of diabetes mellitus on biomechanical properties or fracture healing by observing the histologic changes in the fracture callus in type 1 diabetes animal models. Many of these studies have used streptozotocin to induce experimental diabetes (15,16). Other studies have investigated the biochemical

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changes caused by diabetes. Those studies indicated that both protein and collagen metabolism are significantly decreased in the presence of diabetes (17–21). Subsequently, insulin has been shown to restore these metabolic abnormalities to almost normal (22–24).

Fracture healing and complications in the general diabetic population might be part of the spectrum of bone pathologic features in diabetes, of which Charcot neuroarthropathy represents end-stage disease. Most animal studies have evaluated young animals before diabetes-related complications have developed. Thus, it is the presence and severity of diabetes-related comorbidities (peripheral neuropathy and peripheral arterial disease) in humans, rather than the diabetes itself, that have been shown to increase the risk of complications (25,26).

Several drugs used to control diabetes have been implicated to have adverse effects on the skeleton (27,28). Metformin is a commonly used drug to reduce the blood glucose level in patients with T2DM; however, its role in fracture healing is not clear. Two studies have reported that metformin is osteogenic in vitro (29,30) but that it has no effect on the osteogenic differentiation of bone marrow-derived mesenchymal stem cells and matrix mineralization (31,32). The purpose of the present study was to evaluate the role of diabetes on fracture healing in a rat femur repair model and to determine whether the treatment of metformin had an effect on the fracture healing in rats with hyperglycemia.

## Materials and Methods

### *Diabetes Model*

Male Zucker diabetic fatty (ZDF) rats, age-matched lean litter mates, 14 weeks old, were obtained from Charles River Laboratories International, Inc. (Wilmington, MA). ZDF animals are an inbred rat model that closely mimics adult-onset diabetes in humans. All male ZDF rats develop impaired glucose tolerance, hyperinsulinemia, insulin resistance, obesity, and hyperlipidemia. Glucose intolerance is observed at about 7 weeks, and the rats are fully diabetic by 8 to 12 weeks (20,23). Zucker obese rats are the parent strain and exhibits insulin resistance, hypertension, dyslipidemia, and vascular dysfunction (33). All the rats were fed Purina rat chow (LabDiet Formulab 5008, Nestlé Purina PetCare Co., Richmond, IN) ad libitum and had free access to water. Glucose checks were performed daily, and the fasting blood glucose was measured weekly. The 36 lean and ZDF rats used in the present study were subdivided into 3 groups: (1) 12 lean rats as the control group (lean group); (2) 12 diabetic rats without blood glucose control (DM group); and (3) 12 diabetic rats treated with 300 mg/kg metformin to reduce the blood glucose levels (DM + Met group). Metformin was administered by a veterinarian for glucose control in the DM + Met group. The metformin was dissolved in drinking water and administered orally. The metformin concentrations were readjusted according to the water consumption after an approximately 2-week evaluation period. The body weights were measured at study onset and at weekly intervals. Animal recovery, activities, and blood glucose were monitored daily and the body weight was measured every 3 to 5 days. Radiographs were taken every week to determine the presence of bone repair or delayed union. All the rats were killed at 6 weeks after surgery to determine the serum biomarker levels and biomechanical properties, which were evaluated using 3-point bending at the midshaft diaphysis to determine stiffness, fracture load, ultimate strength, and fracture load/weight.

### *Measures of Neuropathy*

Von Frey monofilaments were used to evaluate neuropathy by observing the frequency of mechanical withdrawal using calibrated 4-, 10-, and 26-g monofilaments at 2, 4, and 6 weeks. The rats were placed in a poly(methyl methacrylate) cage with holes in the bottom. Each paw was tested 3 times for 1 second or until the monofilament bent. Withdrawal was graded from 0 (no withdrawal) to 2 (maximum) for each probe (19,34).

### *Creation of Femur Fracture and Repair*

All rats underwent stabilization of the right femur using the established technique of intramedullary pinning with Kirschner (K)-wire. A medial parapatellar incision was used. The femural canal was reamed by introducing a 0.062-in. K-wire through the intercondylar notch. This technique provided stabilization during and after the fracture. Immediately after stabilization, the right femur was subjected to a deforming force using a guillotine-style blunt-fracture blade. The closed method has been well described and used by others (35,36). In all the rats, a 0.062-in. K-wire was used to ream the femoral canal, and it was replaced by a smaller diameter, 0.045-in. smooth K-wire. Radiographs were taken weekly to determine the status of fracture healing until termination at 6 weeks.

### *Biomechanical Testing of Sham/Control and Fractured, Repaired Femurs*

Three-point bending of the midshaft diaphyseal portion of normal and fractured, repaired femurs was used to evaluate the structural properties of the femurs in the study groups. This test is performed by loading the top of the specimen while supporting both ends. The femur marked for biomechanical testing was removed, wrapped in gauze, wetted with normal saline, and frozen at −80°C. The test gauge length, the distance between the supports, was held with a constant distance of 20 mm. The femur was compressed at midway between the end supports with an edged tip at the rate of 0.25 mm/s in displacement control (no. 5565; Instron®, High Wycombe, UK). Force and displacement information was collected at a sampling rate of 75 Hz, as previously described (15). The experiment was stopped after the bone fractured. The cross-section of the bone was prepared. Using a digital scanner, digital images were made from the cross-sections and measured with ImageJ software (National Institutes of Health, Bethesda, MD) to determine the dimensions of each bone. The biomechanical properties were then calculated from the force displacement curves. The fracture load is the maximum force (N) recorded in the test. The bending stiffness is the slope from the linear range of the force displacement curve (N/mm). The yield Load (N) is the load that induced plastic deformation in the bone. The fracture energy (mJ) is the energy needed to induce bone fracture. The fracture load per body weight and yield load per body weight were also calculated to normalize the effect of the body weight.

### *Statistical Analysis*

One-way and two-way analysis of variance with Fisher's least significant difference post hoc test were performed using Excel, version 6.0 (Microsoft Corp., Redmond, WA) or Systat, version 10.2 (Systat Software, Chicago, IL) to determine the differences in the compare bending stiffness, yield force, fracture load, and fracture energy between groups. The linear regression analysis was performed to determine the correlation between fracture load and body weight using Systat. All the values are expressed as the mean ± standard error of the mean. The significance level was set at $p = .05$.

## Results

### *Metformin Decreased Weight and Blood Glucose in Diabetic Rats*

The average weight of the rats in the DM group (weight 0.374 to 0.386 kg) was significantly greater than that of the lean group (weight 0.31 to 0.33 kg) at all time points, as expected ($p < .001$ for all). The rats treated with metformin (DM + Met group) in the mid- and late stages weighed less than did the DM group ($p = .024$ and $p = .018$, respectively; Fig. 1). Also, an increase in weight occurred between the mid- and late stages in all groups, probably resulting from the combinational effect of the recovery from the surgery and the continuous growth of the rats. The blood glucose level in the DM group was 2.9- to 3.1-fold (range 391 to 408 mg/dL) greater than that in the lean groups at all time points ($p < .001$; Fig. 2). Similarly, 27.9% of the lower glucose levels were found in the mid- and late stages of the rats treated with metformin. Together, our data suggest that metformin treatment effectively reduces the blood glucose level and weight at 12 days postoperatively (DM + Met versus DM).

### *Delayed Healing in the DM and DM + Met Groups*

From the radiographs, a delay in fracture healing, especially at the 3- to 5-week point, was found in the DM and DM + Met groups (42% and 50%, respectively) compared with the lean group (Fig. 3). No differences were found between DM and DM + Met groups.

### *Decrease Biomechanical Properties in DM and DM + Met Groups*

The results were consistent with significant decreases in the biomechanical properties in the DM groups. Although no differences were found in bending stiffness between the lean and DM groups, a significant decrease in the fracture load was found in the DM group compared with that in the lean group ($p = .022$). A trend toward a decrease was also found in the sham-operated group ($p = .087$; Fig. 4*A*). To our surprise, a decrease in the fracture load was also found in the DM + Met (compared with the DM) repaired femurs (44.8%;