



REVIEW ARTICLE

Impact of Diabetes on Fracture Healing

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Diabetes mellitus is a metabolic disorder associated with several complications, including impaired healing. Bone, as important skeletal structure in the body, is affected by the diabetic condition, particularly during fracture healing processes. In this review, we discuss the normal fracture process, as well as mechanical, histological, and molecular negative changes that take place during diabetic fracture repair. Although diabetes affects cartilages and bones from anabolic aspects, our studies have shed light on the impact of diabetes on the catabolic aspect as well. Several underlying proposed mechanisms involved in impaired diabetic fracture healing were also reviewed. Current potential therapeutic agents, which may improve the healing process, were reviewed based on the current understanding of diabetes' impact on the various stages of fracture healing.

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1. Diabetes, Bone Mineral Density, and Risk of Fracture

Diabetes mellitus is estimated to affect 171 million persons, a figure that is expected to rise to 366 million in 2030.¹ Diabetes causes complications, including osteopenia associated with decreased bone mineral density.^{2–7} Krakauer et al⁸ have suggested that patients with diabetes have reduced bone formation and accumulation during growth, whereas later in life, hyperglycemia leads to increased bone resorption and osteopenia. The effect of osteopenia is thought to contribute significantly to the increased risk of fractures, as evidenced by increase in the risk of hip fracture by at least sixfold,^{4,9} as well as increased incidence of long-bone fractures in diabetics.^{4,10–12} Tuominen et al² reported that low-energy fractures were more common in women with Type 1 diabetes. Recent reviews report that both Type 1 and 2 diabetes are associated with increased fracture risk, as evidenced by recent meta-analyses and cohort studies, and suggest that factors other than decreased bone mineral density, such as hyperglycemia or vascular changes, may contribute to the greater risk.^{13,14} An association among poor glycemic control, as measured by HbA1 levels; bone mineral density; and fractures has also been reported.¹⁵ Studies examining bones in streptozotocin-induced diabetic mice or rats show that there is a significant decrease in bone mineral density, bone mineral

content, and serum osteocalcin levels, and biomechanical strength in the long bones is diminished.^{16–18}

2. Stages of Normal Fracture Healing

The fracture repair process consists of a well-orchestrated series of events that lead to the formation and remodeling of the fracture callus (Table 1¹⁹). As a result of the fracture and interruption of the vasculature at the fracture site, a hematoma develops immediately after injury, which is a source of growth factors that can regulate cellular proliferation and differentiation.²⁰ The initial events are stimulated by an inflammatory response to injury that leads to the recruitment of mesenchymal cells.^{19,21} Mesenchymal cells proliferate and differentiate into chondrogenic or osteogenic lineages in response to bone morphogenetic proteins (BMPs). The periosteum, soft tissue, and bone marrow spaces are potential sources of cells that undergo differentiation. These cells differentiate into chondrocytes that produce cartilage, which helps to mechanically stabilize the fracture callus. After cartilage matrix is formed and mineralized, osteoclasts are generated that cause the resorption of cartilage. During this process, angiogenesis occurs and osteoblasts are formed, leading to subsequent transition from cartilage to bone.¹⁹ The primary bone that is formed is then remodeled and reshaped. Interestingly, inflammatory cytokines play an important role in the initial phase of fracture repair and later when cartilage is degraded.¹⁹ Pro-osteoclastogenic factors, such as tumor necrosis factor (TNF)- α , macrophage colony stimulating factor (MCSF), and Receptor Activator of nuclear factor kappa- B ligand (RANKL), peak

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Table 1 Stages of fracture repair

Stage of fracture repair	Cellular events
Inflammation	Hematoma Inflammation Recruitment of mesenchymal stem cells
Cartilage formation and periosteal response	Chondrogenesis and endochondral ossification begins Cell proliferation in intramembranous ossification Vascular ingrowth Neoangiogenesis
Cartilage resorption and primary bone formation	Phase of most active osteogenesis Bone cell recruitment and woven bone formation Chondrocyte apoptosis and matrix proteolysis Osteoclast formation and cartilage resorption Neoangiogenesis
Secondary bone formation and remodeling	Bone remodeling coupled with osteoblast activity Establishment of marrow

Adapted from Ref. 19.

during cartilage resorption and are particularly important during the transition from cartilage to bone.²² The importance of TNF- α in these events can be noted from studies involving the absence of TNF- α receptor signaling where there is significantly reduced expression of pro-restorative cytokines, which leads to a delay in the removal of cartilage.²²

3. Diabetes and Fracture Healing

Diabetes impairs both soft and hard tissue wound healing. Although, Type 1 but not Type 2 diabetes is associated with decreased bone mineral density, both are associated with an increased likelihood of bone fracture.²³ Studies report several contributing factors, such as the prolonged expression of cytokines and chemokines²⁴; the destruction of matrix that is associated with an imbalance between matrix lytic enzymes and their inhibitors²⁵; as well as defects in reparative capacity because of diminished production of growth and angiogenic factors, decreased proliferation, and increased apoptosis.²⁶ Diabetes impairs fracture healing of bone, including the mandible, hip, and long bones.^{13,23,27,28} Clinical studies have reported delayed union or increased healing time in diabetic subjects compared with matched controls.^{29–31} Long-bone fractures of streptozotocin-induced diabetic animals also exhibit changes consistent with impaired healing, including smaller calluses with decreased bone and reduced mechanical strength compared with those of controls.^{32–34} Evidence that deficits in fracture repair are corrected by treatment with insulin suggests that the effect is the result of diabetes and not a side effect of streptozotocin on bone.^{34,35} Healing long bones in diabetic animals also exhibit reduced strength—a finding supported by delayed recovery of structural and material strength by at least 1 week in the healing femurs of diabetic rats when compared with those of normoglycemic controls.³⁶ In animal models, an approximately 20% decrease in biomechanical strength in femurs and tibias was reported.^{17,18} Other studies demonstrate a twofold reduction in callus mechanical strength and size with decreased bone formation, decreased proliferation, and differentiation of osteoblastic cells during fracture healing in spontaneously or streptozotocin-induced diabetic animals compared with those of matched controls.^{32,33,37–39} The impact of diabetes on the cellular events of fracture healing are summarized in Table 2.

4. Diabetes, Osteoblasts, and Bone Formation

Diabetes reduces the formation of bone, which may be an important mechanism for impaired fracture healing.^{3,6,40,41} Diabetes is also accompanied by decreases in osteocalcin levels, a marker of

Table 2 Changes in fracture healing caused by diabetes

Alterations in diabetic fracture healing
Changes at the tissue level
Reduced bone formation
Reduced cartilage formation
Accelerated loss of cartilage
Reduced vascularity and reduced angiogenesis
Changes at the molecular level
Reduced expression of growth factors
Reduced expression of matrix proteins
Increased expression of proinflammatory genes
Increased expression of pro-osteoclastogenic factors
Increased expression of proapoptotic genes

bone formation.⁴² In Type 1 diabetics, serum alkaline phosphatase (ALP) and osteocalcin levels were significantly lower than those of the control subjects, suggesting reduced bone formation.^{3,40} Other studies have shown that decreased bone formation occurs in some diabetic patients.^{6,40} Streptozotocin-induced diabetic mice and nonobese diabetic mice that spontaneously develop Type 1 diabetes exhibit loss of trabecular bone, increased bone marrow adiposity, and decreased osteocalcin mRNA levels in the tibia.⁴³ *In vitro* studies support this theory as osteoblastic colonies from mesenchymal cells taken from diabetic rats have been shown to decrease in number and size.⁴⁴ In diabetic animals, there is reduced serum level of insulin-like growth factor (IGF-I), dysregulated insulin-like growth factor binding proteins (IGF-BPs).³ Diabetes also has effects on the formation and function of osteoblasts. A study in spontaneously diabetic animal models that evaluated fracture healing at 2, 4, and 6 weeks revealed decreased mineralization, apposition, and timing of mineralization in diabetic rats with poor glucose control.⁴⁵ On a cellular level, hyperglycemia can induce insulin resistance of osteoblasts, which might affect transport and function of glucose transporter 1.⁴⁶ Formation of advanced glycation end products also has been linked to the inhibition of osteoblast function and reduced bone formation.⁴⁷ Taken together, these studies suggested that diabetes decreases the anabolic aspect of fracture healing by affecting osteoblasts in terms of formation, function, and bone deposition.

Although it is known that diabetes affects bone, the exact mechanism is not yet clear. One of the possible underlying processes is high glucose, which, in addition to advanced glycation end products, has been linked to inhibition of osteoblast function and reduced formation of a mineralized matrix *in vitro*.⁴⁸ Treatment of bone marrow stromal cells with high glucose levels reduces proliferation, ALP activity, and the number of bone nodules formed *in vitro*.⁴⁹ One of the major molecular changes in diabetes is the formation of reactive oxygen species (ROS) as a result of high glucose and/or insulin insufficiency or resistance. High levels of ROS have been linked to many diabetic complications.^{50,51} In osteoblasts, ROS has been reported to inhibit differentiation as evidenced by Cbfa1/Runx2, which is a key transcription factor associated with osteoblast differentiation, the reduction of differentiation markers, including ALP, Type I collagen, colony-forming unit—osteoprogenitor formation, and nuclear phosphorylation of Runx2.⁵² Taken together, these studies suggest that high glucose levels are associated with inhibited osteoblast formation or function. Alternatively, the relative absence of insulin rather than the hyperglycemic effects alone may contribute to alterations in bone caused by diabetes.^{38,53}

Diabetes may affect bone through inadequate expression of genes regulating osteoblast differentiation, such as Cbfa1/Runx2 and Dlx5, as reported from multiple low-dose streptozotocin-induced diabetes in animal models.⁵⁴ Another proposed mechanism operating at the transcriptional level is that increase in oxidative stress induces Forkhead box O (FOXO) transcription

factor, which antagonizes Wnt signaling and, thus, decreases bone formation.⁵⁵ These studies may shed light on diabetes influencing processes at the level of gene expression, but further studies are needed to delineate this concept.

Dysfunction in endothelial progenitor cells is one of the proposed mechanisms for diabetic complications.⁵⁶ A study showed that platelet-derived growth factor (PDGF-B) gene transfer improved endothelial progenitor cell recruitment and neovascularization in diabetic wound healing.⁵⁷ Recently, it has been reported that blood flow and microvascular density were reduced in bone marrow isolated from Type 1 diabetic mice, suggesting that microangiopathy might impinge on the integrity of diabetic bone marrow.⁵⁸ Together, these studies suggest that vascular changes in diabetic subjects may impact healing complications.

5. The Effect of Diabetes on Chondrocytes and the Cartilage Phase of Fracture Healing

Diabetes has an effect on the transition from cartilage to bone, which may contribute to impaired fracture healing. Topping et al⁵⁹ reported a 54–70% decrease in Type X collagen in the fracture callus of diabetic rats and suggested that this might have a role in defective diabetic fracture healing. Smaller cartilaginous calluses were reported during healing in streptozotocin-induced diabetic rats, which were accompanied by reduced proliferation of chondroprogenitor cells and chondrocytes in the early stages of healing.⁶⁰ At the molecular level, there were low levels of Type II collagen, Type X collagen, and osteopontin.⁶⁰ Type X collagen expression, chondrocyte maturation, and hypertrophy were also delayed in diabetic experimental models with an associated decrease in mechanical strength.³² A decrease in chondrogenesis and osteogenesis after 7 days was reported in alloxan-induced diabetic rats compared with those in normal controls, which indicated a delay in fracture healing.⁶¹ These studies support a mechanism by which diabetes might affect fracture healing at the stage of cartilage formation.

We investigated how diabetes affected fracture healing by examining repair in a Type 1 model of diabetes induced by multiple low-dose injections of streptozotocin. Studies were carried out on Days 12, 16, and 22, after fracture, to focus on the transition from cartilage to bone.³⁹ On Day 12, fracture callus size and cartilage areas were somewhat larger in the normoglycemic controls as compared with those of the diabetic group, but the difference was not statistically significant. On Day 16, callus size and cartilage area were two- to fourfold larger in the normoglycemic controls as compared with those of the diabetic group. The reduced amount of cartilage in the diabetic group was the result of significantly more callus osteoclasts that resorbed the mineralized cartilage. To examine the underlying mechanisms, mRNA levels of genes that stimulate osteoclast formation were examined. The levels of TNF- α , MCSF, and RANKL were significantly higher in the diabetic group. In addition, mRNAs encoding major aggrecanases that degrade cartilage, ADAMTS 4 and 5, (A disintegrin-like and metallopeptidase with thrombospondin motifs 4 and 5) were higher in healing diabetic fracture calluses. mRNA profiling was also carried out to examine gene sets that may be elevated by diabetes. A total of 38 inflammatory gene sets were examined. The biggest difference between diabetic and normal fracture healing was observed occurred on Day 16 when 31 out of 38 proinflammatory gene sets were upregulated in the diabetic group, including gene sets related to TNF- α and its receptors; interleukins; prostaglandins; and complement, interferon, and inflammatory cells. These differences between diabetic and normal groups were not observed at an earlier (Day 12) or later (Day 22) time point.

Because the cartilage phase is considered a requisite step in the temporal sequence of bone repair,¹⁹ it is likely that alterations in

either the formation or the resorption of cartilage will negatively impact bone repair. This points to a previously unrecognized catabolic event through which diabetes could affect fracture repair by means of increased osteoclastogenesis and resorption of cartilage during the transition from cartilage to bone. This premature loss of cartilage leads to a reduction in callus size and may contribute to decreased bone formation and mechanical strength frequently seen in diabetic fracture healing.

To further examine how diabetes-enhanced inflammation could negatively affect fracture healing, studies were carried out in which TNF- α was blocked by a specific inhibitor.⁶² Increased TNF- α is thought to contribute to a number of diabetic complications, including microangiopathy, neuropathy, cardiovascular diseases, retinopathy, and increased inflammation associated with infection and periodontitis.²⁶ When diabetic mice were treated with the TNF- α -specific inhibitor, pegsunercept, the number of osteoclasts, cartilage loss, and number of TNF- α and RANKL-positive chondrocytes were significantly reduced. This led to increased amount of cartilage in the diabetic group and increased callus size. The results suggest that diabetes enhances TNF- α , which, in turn, leads to dysregulation of inflammatory pathways, greater osteoclast numbers, and greater loss of cartilage in the diabetic fracture calluses. This may negatively impact healing by reducing the anlagen for new bone formation.

Fork head transcription factors of the FOXO subfamily (FOXOs) regulate metabolism, proliferation, and differentiation.⁶³ FOXO1 is activated by oxidative stress and induces the expression of genes that decrease ROS, thereby protecting the cell from oxidative stress.⁶⁴ FOXO1 is upregulated by TNF- α and has been shown to mediate the proapoptotic effects of TNF- α .⁶⁵ It has been proposed that conditions with prolonged or high levels of FOXO1 activation may be deleterious by inducing apoptosis.^{66,67} Because we previously demonstrated that FOXO1 mediated the proapoptotic effects of TNF- α and TNF- α -induced proapoptotic gene expression,⁶⁵ experiments, described later, were undertaken to determine whether TNF- α contributed to impaired fracture healing *in vivo* and whether FOXO1 could potentially regulate mRNA levels of pro-osteoclastogenic factors induced by TNF- α *in vitro*.

FOXO1 knockdown by small interfering RNA (siRNA) significantly reduced TNF- α , RANKL, MCSF, interleukin-1 α , and interleukin-6 mRNA compared with scrambled siRNA. An association between FOXO1 and the TNF- α promoter was demonstrated by ChIP assay (Active Motif, Carlsbad, CA). Moreover, diabetes increased FOXO1 nuclear translocation in chondrocytes *in vivo* and increased FOXO1 DNA-binding activity in diabetic fracture calluses ($p < 0.05$). These results suggest that diabetes-enhanced TNF- α increases the expression of resorptive factors in chondrocytes through a process that involves activation of FOXO1, and that TNF- α dysregulation leads to enhanced osteoclast formation and accelerated loss of cartilage.⁶²

6. Role of Insulin in Bone

Recent evidence suggests that insulin signaling pathways may mediate communication between metabolic control and appropriate bone remodeling.^{68,69} Fulzele et al⁶⁸ demonstrated by means of an *in vitro* model that insulin administration suppresses an inhibitor of osteoblast development, Twist2; enhances expression of osteocalcin; and subsequently promotes bone formation. Ferron et al⁶⁹ showed that insulin signaling in osteoblasts stimulates the production of the inactive form of osteocalcin and promotes osteoclast-mediated bone resorption. Osteoclast activity releases the active form of osteocalcin and promotes glucose homeostasis by increasing sensitivity to insulin.

Insulin has been postulated to elicit an anabolic role in bone.^{53,70,71} However, the insulin receptor (INSR) may not be the

critical receptor in regulating the anabolic response. Increasing data suggest that insulin's anabolic bone effect may occur through alternative receptors, such as the IGF1 receptor, which shares many similar downstream signaling events with the INSR. The INSR is a large transmembrane glycoprotein made up of two α and two β subunits linked by disulfide bonds. The α subunits reside outside the cell and are responsible for insulin binding, whereas the β subunits reside intracellularly and contain tyrosine kinase activity. INSR tyrosine kinase activity is initiated by insulin binding and is greatly enhanced by autophosphorylation. Classically, insulin acting through the INSR is capable of activating two distinct pathways. First is the pathway that culminates in translocation of the glucose transporter 4 to enable cellular glucose uptake. The second pathway increases cell proliferation and cell growth through mitogen-activated protein kinases. These mitogen-activated protein kinases include Erk1/Erk2.⁵³ Insulin binding sites and glucose uptake have been shown in rat osteosarcoma cells (UMR-106) in response to insulin stimulation.^{72–74} Primary calvarial cultures have also been shown to bind insulin, which stimulates glucose uptake in these cells.⁷⁵ Although insulin binding sites were also detected in the osteoblast-like cell line ROS 17/2.8, no glucose uptake in response to insulin was observed in these cells.⁷⁶ Although the connection between insulin binding and glucose uptake in osteoblasts was reported in these early studies, less is known about the activation of cell proliferation and growth by insulin in cultured osteoblasts.

The effect of insulin on specific phases of fracture healing has been difficult to study directly *in vivo* because its systemic administration can result in severe hypoglycemia. Previous investigators have focused on animals with induced diabetes mellitus. Weiss and Reddi⁷⁷ performed an analysis of ectopic bone formed with demineralized bone matrix and subsequent endochondral ossification in diabetic rats. Histomorphometric analysis revealed a delay in chondrogenesis and a marked reduction in vascular invasion within the decalcified bone matrix of diabetic rats. Nondiabetic bone matrix exhibited osteogenesis by Day 11 and remodeling by Day 14. In contrast, very few osteoblasts were even present by Day 14 in the diabetic bone matrix. Cell proliferation levels in the diabetic bone matrix, quantified by [³H] thymidine uptake, reached only about 35% of nondiabetic controls after 3 days. Systemic injection of insulin adequate to obtain physiological blood glucose levels increased cell proliferation to 81% of the controls, which ameliorated this facet of diabetic osteopathology. They also noted decreased chondrogenesis and mineralization in the diabetic animals relative to nondiabetic controls, which was largely normalized by systemic administration of insulin. These studies affirm the necessity of insulin for normal bone regeneration, but they do not distinguish between direct and indirect effects of insulin.

Experimental studies in a diabetic rat femur fracture model show that local application of insulin to the fracture site in diabetic rats, rather than systemic insulin treatment, is sufficient to improve diabetic fracture healing.³⁸ The data suggest that local insulin delivery restores cell proliferation in the diabetic callus, which ultimately enhances healing. A recent study by Dedania et al⁷⁸ demonstrated improved histomorphometric and radiographic parameters of allograft incorporation into a rat femur defect when the defect site was treated with locally applied insulin. The locally applied insulin led to significantly higher percentages of mineralized tissue within the endosteal and gap regions of the femur defect site.⁷⁸ This finding implies that local insulin application may alter the mitogenic activity within the osseous defect site and is a potential alternative to standard treatment options for defect healing or allograft incorporation.

Further research to investigate the potential of local insulin delivery using higher-order animal and human models will

elucidate both the mechanism and efficacy of local insulin administration at the fracture site, to accelerate diabetic bone fracture and nondiabetic bone defect healing.

7. Current Treatment Influence on Bone and Cartilage Formation

Controlled insulin therapy may reverse the impairment in fracture repair in diabetic patients with poor metabolic control.^{32,37,79} Localized insulin therapy improved fracture healing in diabetic animal models in terms of chondrogenesis and cellular proliferation.³⁸ Moreover, treatment of diabetic animals with subcutaneous, controlled-release insulin implants that normalized glucose homeostasis, resulted in normalization of fracture healing.^{37,45} *In vivo* studies from our laboratory showed that at Days 16 and 22 after fracture, when bone formation predominates within the callus, the callus bone area was significantly lesser in diabetic mice than that in mice brought to normoglycemia by insulin treatment.⁸⁰ Other treatments were also implemented focusing on the aspect of bone union, such as that in a study by Kawaguchi et al,⁸¹ which showed that impaired diabetic fracture healing was associated with decreased levels of basic fibroblast growth factor. Application of basic fibroblast growth factor to the fracture site normalized healing in diabetic animals and enhanced repair in normoglycemic animals.⁸¹ Additionally, Coords et al⁸² have recently shown that low-intensity pulsed ultrasound treatment ameliorated reduced growth factor levels, callus cartilage, and callus blood vessel density.

Because diabetes negatively impacts bone healing, attempts to enhance the healing process have been investigated. Platelet-rich plasma (PRP) was investigated as a potential treatment agent for improving diabetic fracture repair because PRP contains high levels of mitogenic growth factors.³⁸ Percutaneous injection of PRP into the fracture site increased cellular proliferation in diabetic rat fracture calluses to a level that matched the level in nondiabetic animals.⁸³ Furthermore, PRP treatment improved the mechanical strength of the diabetic rat fracture calluses. A recent study by Azad et al⁸⁴ reported that recombinant human bone morphogenetic protein-2 (rhBMP-2) enhanced bone formation radiographically and histologically in spontaneously diabetic BB Wistar rats, possibly through an increase in angiogenesis. Mechanically, fracture calluses from diabetic rats treated with rhBMP-2 showed better torque to failure and torsional rigidity values.⁸⁴ Platelet-derived growth factor was also one of the factors investigated in early diabetic fracture callus compared with that of healthy controls.⁸⁵ It has been reported that PDGF was decreased at the mRNA level and protein levels in spontaneously diabetic animals.⁸⁵ Al-Zube et al⁸⁶ investigated the effect of direct application of recombinant human PDGF-BB (rhPDGF-BB) to femur fracture sites in diabetic BB Wistar rats. They reported elevated cell proliferation and increased bone formation in animals treated with rhPDGF compared with those of vehicle or untreated rats. These results indicate that therapeutic approaches can be developed to overcome the negative consequences of diabetes on fracture healing.

In summary, diabetic fracture healing is characterized by anabolic and catabolic changes that contribute to impaired healing. A better understanding of how diabetes, hyperglycemia, hypoglycemia, or insulin resistance affect bone will provide insight into new treatment modalities to enhance diabetic fracture healing.

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