The Pathobiology of Diabetes Mellitus in Bone Metabolism, Fracture Healing, and Complications

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Authors:
Forslund JM Archdeacon MT

Author Affiliation | Disclosures

Johan M. Forslund, BS, MS, and Michael T. Archdeacon, MD, MSE

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Diabetes mellitus (DM) affects a significant portion of the world’s people, and the problem is increasing in magnitude as the population ages and becomes more obese. An estimated 347 million people have diabetes. In the United States, 26 million (roughly 8% of the population) are affected, making DM a major health issue. Given the prevalence of diabetes in the general population, it is not surprising that increasing numbers of fracture patients have DM. Unfortunately, for these patients, many relatively simple fractures can have disastrous outcomes. Infections and wound complications occur in disproportionate numbers, healing time is delayed, and risk for nonunion or malunion is substantially higher.

It is imperative to understand the pathophysiology of DM to appreciate potential interventions and strategies aimed at decreasing complications and improving outcomes of fractures in patients with the disease. In type 1 DM (T1DM), autoimmune destruction of the insulin-secreting β cells in the pancreas results in a complete absence of insulin. Patients with T1DM are dependent on exogenous insulin, and, despite hyperglycemia, most cells in the body are starved for energy. This leads to a catabolic condition, high lipid and protein metabolism, and, in many cases, ketoacidosis. When insulin resistance develops, the β cells are forced to secrete large amounts of insulin; when they fail to keep up, type 2 DM (T2DM) develops. T2DM is often associated with obesity, as excess adipose tissue leads to insulin resistance. Although exogenous insulin may be necessary to treat advanced T2DM, other medications are commonly used to effectively lower blood glucose: Secretagogues (eg, sulfonylureas) facilitate insulin release from β cells, and sensitizers (eg, metformin) increase insulin sensitivity.

The potential morbidity of fractures in patients with DM can be appreciated with the example of ankle fractures. These typically uncomplicated fractures can have very poor outcomes in the setting of DM. In a prospective study of approximately 1500 patients with ankle fractures treated with open reduction and internal fixation, Wukich and colleagues found that 9.5% of patients with DM (vs 2.4% of patients without DM) developed surgical site infections. As defined by Jones and colleagues, major complications of treating ankle fractures in patients with DM include infection, malunion, nonunion, Charcot arthropathy, and amputation. The authors reported major complications in 31% and 17% of patients with and without DM, respectively. Highlighting the importance of glycemic control, Wukich and colleagues found relative risks of 3.8 for infection, 3.4 for noninfectious complications, and 5.0 for revision in complicated (vs uncomplicated) fractures in patients with DM.
Bone Metabolism and Fracture Healing in Diabetes

Insulin appears to play a role in bone metabolism and fracture healing. Therefore, absence of insulin in T1DM and elevated insulin levels associated with T2DM likely influence these metabolic and fracture-healing processes. Insulin has been hypothesized to have an anabolic effect on bone, and in both human and animal models bone mineral density (BMD) is significantly lower in T1DM. Furthermore, BMD in T2DM has been shown to be normal or even elevated. Other metabolic effects of insulin on bone metabolism and growth include slower growth rates and lower BMD in pediatric patients with T1DM versus patients without diabetes, and some animal models show bone microarchitecture altered in the absence of insulin (and reversible with insulin supplementation). These factors seem to contradict the markedly elevated risk for osteoporotic fracture in patients with T2DM, but the mechanisms responsible for this have not been elucidated.

In terms of fracture healing, resorption of cartilage during transition to hard callus appears to be influenced by diabetes. It has been hypothesized that the smaller callus observed in diabetic mice may be secondary to upregulation of osteoclasts. Initial callus size appears not to differ between mice with streptozotocin-induced diabetes, which exhibit a complete absence of insulin, and control mice, but levels of osteoclast and osteoclastogenesis mediators were significantly higher in the diabetic mice. Some investigators think that the reduction in cartilage callus size in diabetic mice is caused by altered mRNA expression and collagen production. Diabetic mice, in addition to showing increased resorption by osteoclasts, demonstrate increased chondrocyte apoptosis, which is thought to activate cartilage resorption events. Exogenous insulin effectively reverses this cartilage loss to baseline levels.

Osteoblasts are a crucial component of the fracture-healing cascade, and acute and chronic hyperglycemia, the hallmark of diabetes, has a variety of effects on osteoblasts. Genes for cell-signal proteins such as osteocalcin, MMP-13, and vascular endothelial growth factor are downregulated in the presence of chronic hyperglycemia, whereas genes for alkaline phosphate are upregulated. Acute hyperglycemia by way of hyperosmolarity is associated with MMP-13 downregulation. Thus, osteoblasts appear to respond to hyperglycemia through 2 different processes: Hyperosmolarity, through osteoblast cell shrinkage, influences the acute response, and hyperglycemia itself, through pathways such as nonenzymatic glycosylation, protein kinase C (PKC) signaling, and the polyl pathway, is the force behind the chronic response. The lineage of osteoblasts from mesenchymal stem cells also can be affected by hyperglycemia, with lower growth rates for mesenchymal stem cells and preferential development toward the adipocyte lineage, while the osteoblast and chondrocyte lineages are downregulated.

Increased osteoblast apoptosis has been associated with diabetes through advanced glycation end-products (AGEs), which modify the structure and function of bioactive compounds through AGE receptors that cross-link and bond to amino groups on bioactive molecules. It has been reported that AGEs interfere with osteoblast development and collagen and osteocalcin production. A common AGE, carboxymethyl lysine-modified collagen,
has been associated with a significant increase in apoptosis through the mitogen-activated protein kinase (MAPK) pathway. Although most of the literature suggests that osteoblast apoptosis is activated by hypoxia, nitric oxide, or integrins, these factors all have the MAPK pathway in common.18

Osteoclasts are also influenced by diabetes. Recent work in T1DM demonstrated that osteoclasts are hyperactive and more sensitive to receptor activator of nuclear factor kB ligand (RANKL) compared with osteoclasts from the population without diabetes. It is also known that osteoclasts are under the control of immunologic mediators like lipopolysaccharide (LPS), a surface component of gram-negative bacteria, and various other proinflammatory cytokines. In patients with diabetes, osteoclasts react differently to LPS and other proinflammatory cytokines, at times with opposing effects, including secretion of RANKL to stimulate resorption by the osteoclast, and precursors preventing progression into osteoclasts. In healthy people, high LPS levels not only prevent precursors from producing more osteoclasts, but promote them to mature into immune-like cells that actually phagocytose bacteria. So, in a state of infection, precursors shift from bone-resorbing osteoclasts to protective immune cells. This phenomenon does not occur in patients with diabetes, in whom the osteoclasts instead resorb more bone and stimulate inflammation by releasing cytokines.19

Interestingly, osteoblasts and osteoclasts are also affected by medications commonly used to treat diabetes. Thiazolidinediones are a class of sensitizers often used to treat patients with T2DM. Thiazolidinediones, particularly rosiglitazone, have been associated with increased bone loss primarily caused by increased bone resorption by osteoclasts.20 In addition, some investigators think that thiazolidinediones induce osteocyte apoptosis, contributing to impaired bone growth.8 Metformin, an insulin sensitizer, appears to have a positive effect on bone growth and fracture risk by enhancing osteoblastogenesis and inhibiting osteoclastogenesis, leading to a protective effect on bone.8

Peripheral neuropathy, which is often associated with diabetes, appears to play a major role in fracture-healing complications, even more so than hyperglycemia does. A recent clinical paper found that patients with diabetic neuropathy had a 44% risk of foot and ankle fracture-healing complications.21 Regardless of the risk, the pathogenesis of diabetic neuropathy can be caused by several mechanisms. Neural tissue does not require insulin for glucose uptake; therefore, in a state of hyperglycemia, aldose reductase shunts glucose to sorbitol while using protective glutathione and generating reactive oxygen species. This oxidative stress results in nerve damage or neuropathy. Microangiopathy, which we discuss in more detail later, also contributes to the development of neuropathy, through compromised flow of blood to neural tissue.22 Another mechanism contributing to diabetic neuropathy involves PKC, which is activated by 1,2-diacylglycerol in the presence of glucose, leading to vascular changes that restrict the flow of blood to peripheral nerves.23 Finally, AGEs may also participate by altering nerve function after binding to neural tissue.

Charcot neuroarthropathy is a complication associated with diabetes, particularly after injury in which chronic inflammation results in damage to the joint through fracture, dislocation, and osteolytic bony destruction. The pathophysiology is attributed to repeated microtrauma caused by loss of protective sensibility and hyperemia caused by dysregulation.24 Sympathetic and sensory nerve fibers are associated with bone, but a few serve as mechanoreceptors and nociceptors, which can activate substance P, calcitonin gene-related peptide, and vasoactive intestinal peptide—neuropeptides all thought to be involved in the inflammatory process, and in the activation of osteoblasts and osteoclasts. In diabetic neuropathy, many of these neuropeptides show a reduced regulation response, which can lead to impaired fracture healing. In particular, osteoclast activity is upregulated, and consequently bone resorption is increased. In addition to the neuropeptides mentioned, RANKL is one mechanism by which this upregulation occurs.25

It is clear that bone metabolism and fracture healing are complex processes. In the patient with diabetes, many
factors are affected, including BMD, bone microarchitecture and bone growth, cartilage resorption during callus formation, osteoblast and osteoclast activation through both altered responses to cell signals and pharmacologic interactions, and, finally, peripheral neuropathy. Given the complex interactions described, it is likely that these factors in combination, as well as those yet undiscovered, negatively affect fracture healing.

**Wound Healing and Vasculopathy in Diabetes**

Bone healing and soft-tissue healing depend on many of the same factors. Therefore, interactions between neuropathy and vasculopathy can have a tremendous influence on wound healing in patients with diabetes. The vascular pathology that occurs in diabetes depends in part on the fact that endothelial cells do not require insulin for glucose uptake and therefore are more susceptible to damage by hyperglycemia. As already discussed, shunting of glucose through the polyol pathway with the resultant oxidative stress is partly responsible for angiopathy in diabetes.

Also as already discussed, AGEs affect intracellular processes by protein binding and gene regulation and by disrupting the communication between cells and the surrounding matrix. From an extracellular standpoint, AGEs bind to circulating proteins, promoting inflammation and upregulation/downregulation of growth factors, including endothelial nitric oxide synthase, a critical vasodilator. Endothelin 1, on the other hand, is a potent vasoconstrictor. It is upregulated while transforming growth factor β and plasminogen activator inhibitor 1 are upregulated, resulting in further vascular damage. The common mechanism for this vasculopathy appears to be superoxide production in the mitochondria, caused by excess glucose oxidation forcing coenzyme Q to donate electrons to oxygen, producing the superoxides. Superoxides in turn inhibit glyceraldehyde 3-phosphate dehydrogenase, which activates the polyol pathway, AGE formation, PKC, and the hexosamine pathway. In addition to coenzyme Q, several other enzymes generate reactive oxygen species, including nicotinamide adenine dinucleotide phosphate oxidase, aldehyde oxidase, xanthine oxidase, and glucose oxidase. These reactive oxygen species exacerbate oxidative stress, leading to further endothelial cell damage, and cause vascular smooth muscle injury.

Further influencing the wound-healing environment are the effects of diabetes on blood vessel maintenance and repair as well as angiogenesis in response to local-tissue hypoxia. Vessel-repair mechanisms require endothelial progenitor cells (EPCs), which are released in response to cytokines and neural impulses. Bone marrow-derived EPCs have inadequate proliferative and migratory ability in patients with diabetes. In a diabetic mouse model, EPCs appear in the bone marrow at normal levels, but levels in circulation are lower than anticipated, because of poor proliferation and mobilization, it is thought. In terms of local-tissue hypoxia, hypoxia-inducible factor 1 (HIF-1) is an important transcription factor that promotes the expression of genes that in turn induce angiogenesis. The mechanism of this response is complex, and hyperglycemia has the potential to interfere in various steps of the cycle. In response to local-tissue hypoxia, the HIF-1α subunit must localize to the target site, where it combines with HIF-1β to create the active dimer, HIF-1. This active dimer is regulated through degradation of the α subunit in the presence of normal oxygen levels. However, in a state of hypoxia, the molecule is stabilized, promoting angiogenesis and fibroblast migration. Recent evidence suggests that hyperglycemia interferes with the dimerization process and that there is a failure of HIF-1α to locate into the nucleus, which is
crucial for gene upregulation.\textsuperscript{31,33}

### Infection in Diabetes

Throughout the literature, the risk for infection after fracture is consistently higher in patients with diabetes than without diabetes. There likely are many contributing factors, including diminished blood flow and vasculopathy as well as a dampened immune response as a result of defective granulocytic, phagocytic, and chemotactic functions and defective macrophagic activity. Typically, polymorphonuclear leukocytes (PMNs) migrate to bacteria and initiate bacteriocidal activity, and then macrophages phagocytize PMNs and other damaged cells. PMNs demonstrate impaired function in patients with diabetes—reduced phagocytic response and respiratory burst as well as chemotaxis impairment. The diminished phagocytic potential is substantial, with experiments showing an almost 50% reduction in ingestion of \textit{Staphylococcus aureus} in a patient with diabetes than in one without diabetes.\textsuperscript{34} Expression of surface integrins, which mediate PMN adhesion to the basement membrane of the tissue, appears to be negatively altered in both T1DM and T2DM, furthering diminishing the chemotactic response of PMNs.\textsuperscript{35} Impaired leukocyte function may also be a downstream effect of vasculopathy and associated hypoxia/hypoxemia as PMNs use superoxide radicals and other oxidizing agents to create a bacteriocidal environment that is negatively impacted in a low oxygen state.\textsuperscript{3} In addition, macrophages are disabled in patients with diabetes. (In rats with streptozotocin-induced diabetes, there is inadequate activation of macrophages in the early stages of healing.\textsuperscript{36}) Furthermore, AGEs similar to those mentioned earlier have a significant negative impact on macrophagic function.\textsuperscript{37} Thus, both the activation and the activity of macrophages appear to be impeded in the setting of diabetes.

### Potential New Treatment Modalities

There is tremendous potential for clinical intervention to prevent pathologic outcomes in patients with diabetes, given the complex tissue, cellular, and molecular interactions, particularly those caused by hyperglycemia. At the bone tissue level, increased osteoclastic activity in patients with diabetes has been associated with many complications, including Charcot arthropathy. RANKL modulates differentiation and activation of osteoclasts; thus, RANKL inhibition is a possible therapeutic target.\textsuperscript{38} Elevated AGE levels have also been observed in patients with Charcot arthropathy, and RAGE, the receptor for AGE, has been seen at lower than expected levels in patients with diabetes. RAGE appears to provide a protective effect against excessive bone resorption; therefore, treatment that increases RAGE levels—such as angiotensin-converting-enzyme inhibitors, statins, and glitazones—may be capable of mitigating the osteoclastic effects in Charcot arthropathy.\textsuperscript{39}
AGE formation appears to be central to many pathologic processes in diabetes, so it is a logical therapeutic target, particularly for pathologic processes at the vascular tissue level. Aminoguanidine is an anti-AGE agent that was initially used to prevent diabetic retinopathy, but it has also been shown to prevent general vascular complications in diabetic animal models. The terminal amino residue in the compound specifically binds glucose-derived reactive intermediates and prevents cross-linking, which renders them inactive. Disrupting those cross-links is another treatment strategy. N-phenacylthiazolium bromide and 3-phenacyl-4,5-dimethylthiazolium chloride (ALT-711 or alagebrium) are compounds that have been shown to break cross-links in a diabetic rat model.\(^\text{16}\)

Another tactic for reducing vascular pathology involves mitigating superoxide radicals, as these radicals are generated from the glycolytic intermediates in hyperglycemic states. It has been reasoned that, if the concentration of these intermediates can be decreased, there would be less substrate available for the pathways that lead to radical formation. One approach is to use transketolase, an enzyme that shunts intermediates to pathways that do not produce superoxide radicals. In the treatment of patients with diabetic retinopathy, early data appear promising with benfotiamine, a thiamine derivative, which upregulates transketolase 250%. An additional tactic involves catalytic antioxidants—namely, superoxide dismutase/catalase mimetic, which has been shown to reduce hyperglycemia-induced superoxides. These interventions are appealing because of their nonstoichiometric reactions, which render them potentially more potent antioxidants.\(^\text{26}\)

Potential neurologic interventions include recombinant human nerve growth factor, neurotrophic factors, and gene therapy, all directed toward preventing or regenerating neuropathic tissues in patients with diabetes. Most of these interventions, however, remain theoretical. Few trials have demonstrated clinically significant improvement. In patients with T1DM, however, the absence of circulating C-peptide is thought to contribute to diabetic neuropathy. Results of trials with subcutaneous C-peptide treatment suggest improvement in both sural sensory and vibration perception after only 12 weeks.\(^\text{40}\) These novel treatments further emphasize the potential for intervention at the tissue, cellular, and molecular levels.

**Conclusion**

Whereas most fractures are uncomplicated in healthy patients, they can have devastating consequences in patients with diabetes. In this review, we have highlighted many of the pathologic processes that can influence outcomes of fractures in patients with diabetes. These problems will become more common as the population ages, age-related risks for osteoporosis and fragility fracture increase, and diabetes becomes nearly epidemic in our increasingly obese, sedentary society. Although some progress has been made, a more thorough intervention strategy is needed to improve both bone and soft-tissue outcomes of fractures in patients with diabetes.

**Key Info**
Figures/Tables

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