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Diabetes and Its Effect on Bone and Fracture Healing

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Abstract

Diabetes mellitus is a metabolic disorder that increases fracture risk and interferes with bone formation and impairs fracture healing. Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) both increase fracture risk and have several common features that affect bone including hyperglycemia and increased AGE formation, ROS generation, and inflammation. These factors affect both osteoblasts and osteoclasts lead to increased osteoclasts and reduced numbers of osteoblasts and bone formation. In addition to fracture healing, T1DM and T2DM impair bone formation under conditions of perturbation such as bacteria induced periodontal bone loss, which reduces expression of factors that stimulate osteoblasts such as BMPs and growth factors and increase osteoblast apoptosis.

Keywords

diabetes; inflammation; advanced glycation end-products (AGE); oxidative stress; Insulin; Hyperglycemia; osteoblast; osteoclast; fracture healing

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disease with high blood glucose levels [1-3]. Diabetes results from deficits in the production of insulin or deficit insulin resistance coupled with insufficient insulin production. Type 1 diabetes mellitus (T1DM) is due to the lack of insulin production by the pancreas and requires daily administration of insulin. It is typically caused by destruction of pancreatic β-cells of autoimmune etiology. Type 2 diabetes mellitus (T2DM) is characterized by the inability to use insulin efficiently, referred to as insulin resistance combined with an inability to produce a sufficient amount of insulin to overcome the insulin resistance. Diabetes mellitus often leads to serious complications that affect the heart, blood vessels, eyes, kidneys, and nerves. It has also been increasingly recognized that diabetes adversely affects bone health.

Insulin receptor signaling activates Ras, which leads to activation of MAP kinases and promotes growth. Insulin induces another intracellular cascade that leads to phosphorylation of insulin receptor substrate 1 (IRS1) and IRS2 and activation of phosphatidylinositide-3-kinase (PI3K), which phosphorylates and activates Akt. One of the effects of Akt is to phosphorylate and deactivate Foxo1; another is to phosphorylate and inhibit glycogen synthase kinase-3β (Gsk3β). FOXO1 is a transcription factor that induces genes that control glycogenolysis and gluconeogenesis and its activity can lead to hyperglycemia. In addition FOXO1 is activated in tissues associated with a number of diabetic complications including soft tissue during wound healing and bone fracture [4, 5]. Insulin resistance may involve reduced expression or phosphorylation of IRS-1/IRS-2 due to various causes including inflammation. Diminished IRS1 and IRS2 activity reduces activation of PI3K but increases MAP kinase activation. Normal expression and function of IRS1 and IRS2 is needed to activate PI3K and Akt. Akt signaling prevents inappropriate activation of FOXO1 and is essential for maintaining homeostasis. Thus, a reduction in insulin signaling leads to reduced Akt and increased FOXO1 activation to promote hyperglycemia. This may contribute to organ failure and diabetic complications due to insulin resistance.

High levels of glucose contribute to diabetic complications by inducing stress at the cellular level, glycating proteins that lead to the formation of advanced glycation endproducts, increasing production of reactive oxygen species, and enhancing expression of cytokines such as tumor necrosis factor [1, 6, 7]. In diabetic humans and animals there is increased production of inflammatory mediators by macrophages in adipose tissue leading to increased systemic inflammation, which among other factors contributes to insulin resistance [8]. Diabetic conditions such as high glucose levels, increased formation of advanced glycation endproducts and increased generation of ROS lead to greater expression of inflammatory cytokines at the local level when tissues are perturbated by events such as wounding.

Diabetes, Inflammation and Bone

Pro-inflammatory mediators including TNF-α, IL-1β, IL-6 and IL-18 are increased locally in diabetes mellitus and are thought to contribute to diabetic complications [7, 9]. Diabetics have difficulty in down regulating inflammation once induced [10, 11]. Increased levels of

TNF may limit the capacity of diabetics to down regulate other inflammatory genes and increase apoptosis, which has been shown to reduce bone coupling in diabetic animals [12].

During perturbation diabetes increases and prolongs inflammation, which may lead to enhanced osteoclastogenesis. Diabetes increases osteoclast formation in a number of conditions including periodontal disease, fracture healing and osteoporosis [6, 12, 13]. Diabetes-increased osteoclasts may pertain to situations where bone is challenged by injury or inflammation rather than basal levels. Diabetic animals with periodontitis have higher levels of IL-1β, TNF-α, and prostaglandin E_2, which induce and prolong osteoclast mediated resorption [14]. Diabetic rats with periodontitis and T1DM have a 2 to 4-fold increase in the number of osteoclasts and individuals with T1DM have increased levels of IL-17 and IL-23, which promote osteoclast formation through RANKL (Figure 1) [15, 16]. T2DM rats have a 2 to 4-fold increase in osteoclasts induced by periodontal infection compared to infected normoglycemic controls [11, 17, 18]. Similarly, humans with T2DM and periodontitis have significantly increased levels of TNF-α, IL-1β and IL-6 associated with prolonged inflammation and increased lipid peroxidation and dyslipidemia [16, 19, 20]. Diabetes leads to increased RANKL/OPG ratios and TNF levels that contribute to greater bone resorption [11, 21]. In humans, the ratio of RANKL/OPG and TNF levels are increased in poorly controlled diabetics [19, 22]. Fatty acid levels in diabetics may also contribute to increased osteoclastogenesis [23]. The capacity to resolve inflammation is an important aspect of limiting bone resorption as shown by diminished bone loss in animals treated with resolvins [24] or by use of TNF inhibitors [10, 11].

Diabetes decreases osteoblast formation and function and reduces the number of osteoblasts. Bone formation is reduced in diabetics as reflected by reduced levels of osteocalcin in type 2 diabetic patients compared to non-diabetic controls, reflecting a decrease in osteoblast activity, which is inversely related to IL-6 and C reactive protein (CRP) [25]. Rats with type 2 diabetes have decreased expression of BMPs and FGF, reduced osteocalcin expression and reduced bone coupling [12]. These deficits are linked to diabetes-increased inflammation since they are reversed by inhibition of TNF [12]. A mechanism through which this may occur is greater or prolonged expression of TNF in bone of diabetics when stimulated by injury or inflammation that leads to increased nuclear factor-kappa-B activity and reduced expression of fra-1 and runx2 in osteoblasts and reduced expression of mediators that stimulate osteoblast growth and differentiation (Figure 2) [26, 27]. Diabetes-enhanced inflammation may reduce osteoblast numbers through increased apoptosis. Type 1 diabetes increases osteoblast caspase 3 activity and Bax/Bcl-2 ratio mediated by increased levels of TNF-α [28]. Type 2 diabetes also increases the expression of pro-apoptotic genes that affect bone [10].

Diabetes, AGEs and Bone

Elevated levels of glucose enhance protein glycation (nonenzymatic glycosylation), with the formation of advanced glycation end-products (AGEs). AGEs are non-enzymatic chemical modifications of proteins by aldose sugars, formed by the oxidation of products generated during the Maillard reaction. The accumulation of AGEs has been associated with diabetic complications as well as degenerative diseases that occur with aging. AGEs bind to a

number of receptors including the receptor for AGEs (RAGE) and stimulate inflammatory cytokines [29]. Diabetes increases formation of AGEs and increases RAGE expression [6]. RAGE signaling activates the transcription factor NF-κB to increase expression of the receptor activator for nuclear factor κ-B ligand (RANKL) [30]. AGEs and hyperglycemia are linked to increased osteoclast formation (Figure 1) [31, 32] and RAGE is expressed in osteoclasts and stimulates osteoclastogenesis [33]. Mice that lack RAGE have decreased bone resorption and increased bone mass [33]. Blockade of RAGE signaling by treatment of mice with soluble RAGE reduces bacteria-induced periodontal bone loss [34]. RAGE also down regulates expression of osteoprotegerin (OPG) to enhance osteoclastogenesis and bone resorption [35]. In addition, AGEs inhibit differentiation of osteoblasts as reflected by reduced expression of alkaline phosphate and collagen 1α1 and inhibited formation of a mineralized matrix [36]. Moreover, there is evidence that AGEs induce osteoblast apoptosis to reduce osteoblast numbers and impair bone formation [37].

Diabetes, ROS and Bone

Under diabetic conditions, various tissues produce reactive oxygen species (ROS) [38, 39]. Oxidative stress is increased in diabetes and contributes to diabetic complications. Superoxide production is increased in the mitochondria as a result of increased glucose levels, which lead to greater inflammation [40, 41]. A primary mechanism is the overproduction of the superoxide anion (O^{-2}) by the mitochondrial electron transport chain. In addition, diabetes causes a reduction in antioxidant levels to increase susceptibility to oxidative stress [42]. There are several sources of ROS in cells including stimulation by AGEs [43], high glucose induced overload of the electron transport chain in mitochondria [40] and the activity of membrane-bound NADPH oxidase [44, 45]. High levels of ROS negatively affect bone [46, 47]. Intracellular H_2O_2 increases the differentiation and survival of osteoclasts. The formation of reactive oxygen species (ROS) induces RANKL expression and enhances greater osteoclast formation [48]. Hyperglycemia-induced ROS production also increases expression of RAGE, which may contribute to osteoclast formation [49].

The long-term effect of oxidative stress is to reduce bone mass. The importance of protection against oxidative stress was shown by deletion of the transcription factor, forkhead box-O (FOXO). Deletion of FOXO1, FOXO3 and FOXO4 results in reduced expression of antioxidant enzymes and failure to protect against oxidative stress [50]. Interestingly, FOXO1 is induced by RANKL stimulation and has a direct effect in stimulating osteoclast formation [51]. The long-term effects of oxidative stress may be particularly important for long-lived cells such as osteocytes and mesenchymal stem cells. Mesenchymal stem cells play an essential role in bone formation and osteocytes are critical for regulating bone remodeling, particularly in response to mechanical stimulation. The long term impact of oxidative stress on bone maybe mediates through its detrimental effect on these two types of long-lived cells [52]. Since diabetes increases formation of superoxide radicals and inhibits antioxidant defenses its impact on mesenchymal stem cells and osteocytes may be one of the mechanisms through which diabetes impacts the long term health of bone.

Diabetes, Hyperglycemia and Bone

Studies on osteoclasts derived from db/db T2DM mice and T2DM patients found that osteoclasts differentiation was enhanced by hyperglycemia, suggesting an increased capacity for bone resorption. This may contribute to increased alveolar bone loss in T2DM patients with periodontitis [31]. High levels of glucose stimulate the generation of reactive oxygen species which in turn can increase osteoclast formation and activity [53, 54]. Since the effect of high glucose often takes several days it is possible that it works indirectly by stimulating increased generation of ROS, increased cytokine expression and formation of AGEs. Increased glucose levels interfere with osteoblast differentiation and osteoblast function reflected by decreased expression of the osteoblast marker genes (Figure 2) [55]. High glucose stimulates production of reactive oxygen species and activation of NF-kB to affect osteoblasts [56]. Hyperglycemia may affect bone through enhanced expression of proinflammatory cytokines such as TNFα, which reduces osteoblast differentiation, osteoblast activity and increases osteoblast apoptosis [57, 58]. High glucose levels reduce expression of the transcription factor RUNX2 and inhibit bone formation [54, 59-61]. Furthermore, it interferes with production of a mineralized matrix [55]. Osteoblast viability is decreased by high glucose. Another mechanism is through increased PPARγ activation that promotes adipogenesis from mesenchymal stem cells at the expense of bone formation to reduce bone mass [62].

Diabetes, Insulin and Bone

Insulin binds to receptors on osteoblasts and stimulates anabolic effects [63]. It is possible that the reduced insulin levels or reduced insulin signaling in osteoblasts negatively affects bone and contributes to reduced bone formation caused by diabetes [64, 65]. Activation of insulin-like substrate1 (IRS-1) affects bone turnover, while activation of IRS-2 shifts the balance of bone formation and resorption towards formation. Insulin stimulates osteoblast proliferation, inactivates p27, and promotes collagen synthesis [66]. In T1DM, the deficiency of insulin and IGF-1 leads to impaired bone formation, abnormal mineralization, abnormal bone microarchitecture, increased fragility of the bone, and reduced peak bone mass [67]. It has been proposed that hyperinsulinia in the early stages of T2DM increases bone mass through effects on bone formation via IRS-1 and IRS-2 surface receptors [68]. Physiological levels of insulin reduce the ability of PTH to activate protein kinase C in osteoblasts [69, 70], suggesting that insulin may be a physiological antagonist of bone resorption. T1DM diabetes and later stages of T2DM reduced insulin signaling may remove a brake on osteoblast-induced osteoclast formation.

Diabetes and Impaired Fracture Healing

Diabetic fracture is a significant co-morbidity of both type I and type II diabetes and is characterized by microarchitectural changes that decrease bone quality [64, 66]. Meta-analysis shows a consistent pattern of increased risk of fracture in men and women and in studies conducted in the United States and Europe. The Nurses' Health Study with 109,983 women aged 34–59 years and follow up 22 years later indicated that both T1DM and T2DM are both associated with an increased risk of hip fracture [71]. The relative risk of hip fracture is increased 6-7 fold for individuals with T1DM, which is considerably higher than

the increased risk (1.4-1.7 fold) in T2DM [72]. The fracture risk of T1DM increases because of a decrease of BMD, which is linked to impaired bone formation that may be linked to a deficiency of insulin and insulin-like growth factor-1 (IGF-1) [73]. T2DM is often characterized by normal or high bone mineral density (BMD). Diabetes may be associated with a reduction of bone strength that is not reflected in the measurement of BMD [74] results in high risk of fracture.

Fracture repair involves formation of a hematoma after injury that generates the production of cytokines and growth factors. This leads to an inflammatory response that is necessary for the recruitment of mesenchymal stem cells [75, 76]. These cells proliferate and differentiate to chondrocytes that form cartilage during the endochondral phase of bone formation. Cells along the periosteum differentiate into osteoblasts to produce new bone. The cartilage mineralizes and mechanically stabilizes the fracture site. Mineralized cartilage is then removed by the action of osteoclasts. Factors important in this process are TNF-α, macrophage colony stimulating factor (MCSF) and RANKL [77]. The transition from cartilage to bone is linked to increased angiogenesis [75]. The last phase is bone remodeling, which involves the action of osteoclasts and osteoblasts to reshape the bone to its final form. Diabetic animals exhibit both decreased and delayed bone formation [78]. Diabetic fracture healing may be caused in part, by reduced growth factor levels as shown by improved healing with application of FGF-2 to the fracture site [79].

Healing of fractures in diabetic patients is prolonged by 87% [80] and has a 3.4 fold higher risk of complications including delayed union, non-union, redislocation or pseudoarthrosis [81, 82]. Clinical studies in humans indicate that diabetes delays fracture healing [82]. A study of spontaneously diabetic animals revealed that diabetic fracture healing was characterized by decreased bone apposition and mineralization [78]. The reparative phase of bone fracture healing is initiated by proliferation and chondroblastic differentiation of periosteal precursor cells resulting in a hyaline cartilage callus around the wounded bone [83]. Imbalances in chondrocyte apoptosis, premature removal of cartilage, reduced osteoblast differentiation and function and alterations in vascularization have been shown to affect the transition from cartilage to bone [84, 85]. Supernormal osteoclast activity disturbs remodeling of the osseous callus [84]. It has been proposed that insulin insufficiency, hyperglycemia and oxidative stress are mechanisms that affect fracture healing in T1DM and T2DM. They may reduce osteoblast differentiation, increase osteoclast activity, and alter apoptosis of chondrocytes and osteoblasts to interfere with fracture healing in diabetic patients [84, 86-88].

Type I collagen is the major protein component in bone. Enzymatic cross-linking between collagen molecules is essential and tightly regulated. Accumulation of AGEs in cortical and trabecular bone increases the stiffness of the collagen network and reduce ductility [89, 90]. These alterations can lead to increased fragility [91]. Non enzymatic glycation causes a significant reduction in propagation fracture toughness and bone [92]. In addition to its structural effects, AGEs can affect the function of bone cells to induce apoptosis, interfere with differentiation and function of osteoblasts and reduce bone mineralization [36, 93].

Insulin acts directly on osteoblasts to increased proliferation, reduce apoptosis, stimulate glucose uptake, increase collagen synthesis and enhance sensitivity to PTH [94, 95]. Insulin also increases chondrocyte proliferation, differentiation, formation of extra cellular matrix [96, 97]. In contrast osteoclast activity in vitro is reduced by insulin [98]. Treatment with systemic insulin reverses impaired fracture healing suggests that insulin signaling plays a necessary role in repair. However the interpretation is limited by the fact that insulin also treats hyperglycemia making it unclear whether the effects are due to the direct effects of insulin on bone cells or the indirect effects due to reversal of hyperglycemia or both [99-101]. Experiments have been performed to study the effect of local insulin application to fracture healing. These studies suggest that insulin has direct effects on the repair process [65]. Local application of insulin restored the deficit in cell proliferation in diabetic animals and improved fracture healing. Histologic and radiographic outcomes of osseous healing in a femoral defect model in diabetic animals were also improved by local insulin delivery with enhanced formation of mineralized tissue at the defect site [102]. In a non-diabetic rat model local insulin accelerated fracture healing but did not alter the final outcome. At early time points application of local insulin increased VEGF expression, enhanced vascularity and increased formation of mineralized tissue and increased mechanical strength at early time points [103].

The complications of diabetes mellitus affect the vasculature in the form of macro- and microangiopathy and wound healing. Both T1DM and T2DM contribute to diabetic macroangiopathy, which leads to greater atherosclerosis and microangiopathy, which contributes to diabetic retinopathy and impaired wound healing [104]. AGEs, defective signal transduction, and an imbalance of matrix metalloproteinases (MMPs) can all lead to the progression of atherosclerosis in major arteries. Increased levels of prothrombotic factors, restricted formation of collateral vessels and increased loss of endothelial cells and pericytes are important aspects of microangiopathy [105]. Moreover, in diabetic wound healing high glucose levels alter the downstream targets of the transcription factor FOXO1 to induce inflammatory mediators instead of TGFβ1, providing an epigenetic explanation for reduced growth factor and increased expression of inflammatory mediators in diabetic wounds [106].

Summary

T1DM and T2DM both increase fracture risk and have several common mechanisms including increased AGE formation, increased ROS generation, and increased inflammation. These factors affect osteoblasts and osteoclasts as summarized in Figures 1 and 2. However there are significant differences whereby T1DM has a greater effect on bone mass and T2DM affects bone quality. Both humans and animal models of T1DM and T2DM display impaired fracture healing but T1DM patients have a greater risk of developing fractures. Moreover, animals with T1DM and T2DM exhibit impaired bone formation under conditions of perturbation such as bacteria induced periodontal bone loss and bone fracture healing.

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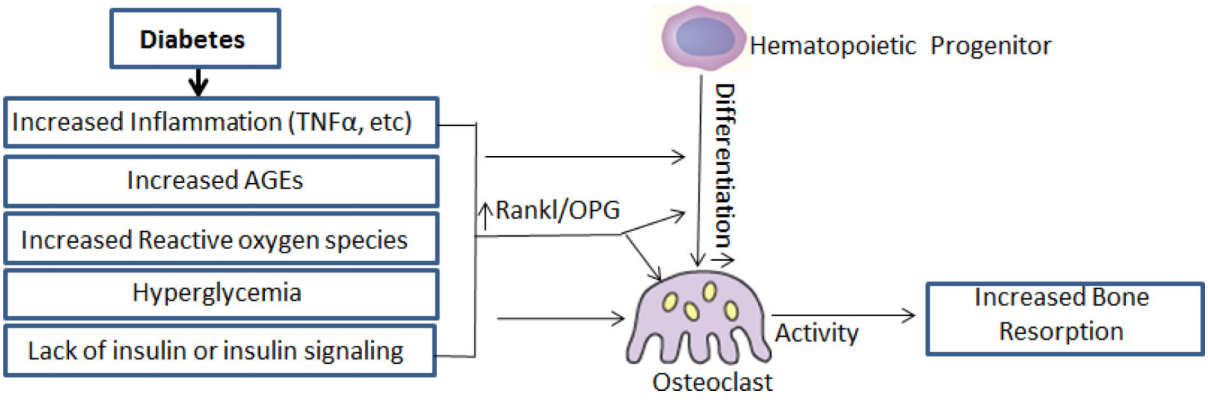

Figure1. Mechanisms of diabetes-increased osteoclastogenesis

Diabetes leads to hyperglycemia, enhanced and prolonged inflammation, formation of AGEs and generation of ROS. This dysregulation as well as reduced insulin signaling may lead to increased osteoclast formation, particularly when bone is challenged by wounding, bacteria induced inflammation or other events that disrupt homeostasis. This dysregulation may lead to an increased RANKL/OPG ratio or affect osteoblasts through other mechanisms to increase bone resorption.

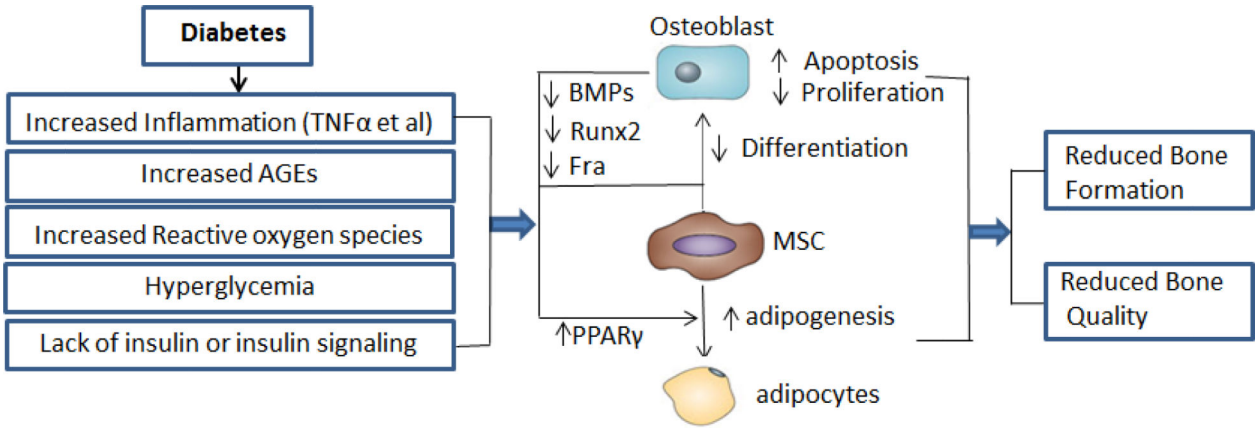

Figure2.

Mechanisms of diabetes-reduced bone formation. Diabetes leads to hyperglycemia, enhanced and prolonged inflammation, formation of AGEs and generation of ROS. This dysregulation as well as reduced insulin signaling may adversely affect osteoblasts and reduce bone formation particularly when bone is challenged by wounding, bacteria induced inflammation or other events that disrupt homeostasis. The effect of dysregulation may lead to a reduction in BMPs, Runx2 or Fra1, an increase in PPARγ or other mechanisms to reduce bone formation or bone quality.