

REVIEW

Update on the impact of type 2 diabetes mellitus on bone metabolism and material properties

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Abstract

The prevalence of type 2 diabetes mellitus (T2DM) is increasing worldwide, especially as a result of our aging society, high caloric intake and sedentary lifestyle. Besides the well-known complications of T2DM on the cardiovascular system, the eyes, kidneys and nerves, bone strength is also impaired in diabetic patients. Patients with T2DM have a 40–70% increased risk for fractures, despite having a normal to increased bone mineral density, suggesting that other factors besides bone quantity must account for increased bone fragility. This review summarizes the current knowledge on the complex effects of T2DM on bone including effects on bone cells, bone material properties and other endocrine systems that subsequently affect bone, discusses the effects of T2DM medications on bone and concludes with a model identifying factors that may contribute to poor bone quality and increased bone fragility in T2DM.

Key Words

- ▶ diabetes
- ▶ bone

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Introduction

The prevalence of diabetes mellitus is increasing worldwide with diabetes-related complications accounting for up to 60–70% of health-care costs related to diabetes (1, 2). Besides the well-known renal and cardiovascular complications, the increased risk for fragility fractures has recently been recognized as an important complication of both type 1 and type 2 diabetes mellitus (T1DM, T2DM) (3, 4, 5). While type 1 diabetics have low bone mineral density and a six- to sevenfold higher risk for fractures, type 2 diabetics have normal to high bone mineral density and up to threefold higher fracture risk (6, 7, 8). Despite the similarity of chronic hyperglycemia, T1DM and T2DM have distinct pathophysiological mechanisms, which may differently affect bone metabolism. In both cases, the underlying mechanisms of poor bone strength are not well understood. Considering that T2DM accounts for the

majority of diabetes cases (>90%), this review will focus on summarizing the current knowledge on mechanisms that contribute to bone fragility in T2DM.

Mechanisms that lead to bone fragility in T2DM are manifold and encompass direct and indirect effects of T2DM on bone. Several studies have shown that T2DM in humans and animals is associated with suppressed bone formation and with negative effects on the mechanosensing properties of osteocytes, while effects on bone resorption are less consistently described. In humans, biochemical markers and bone histomorphometry reveal a low bone turnover in T2DM (9, 10, 11, 12, 13). In animals, however, low bone formation is also a characteristic of T2DM, while bone resorption parameters are usually increased (14, 15, 16, 17, 18). At a structural level, the accumulation of advanced glycation end (AGE) products

under diabetic conditions has been proposed to alter collagen structure and contribute to impaired material properties. Along those lines, patients with T2DM have increased cortical porosity, which may further contribute to reduced bone strength (Fig. 1). Besides these direct effects of high glucose levels on bone, the increased risk of fractures may also be explained by the presence of diabetic complications on the eyes, kidney and nerves, decreased physical activity, lower vitamin D levels and higher risk of falls. In particular microvascular impairment, which may also affect the bone vasculature, and increased bone marrow adiposity (19) (Fig. 1) may be key factors that contribute to skeletal alterations and translate into a higher incidence of fractures, delayed fracture healing and delayed osseointegration.

Structural and material properties of bone in T2DM

Patients with T2DM have a particularly high risk to fracture their hip, wrists and feet, and fracture risk further increases with disease duration, insulin intake and poor control of hyperglycemia (6, 20, 21, 22). While in postmenopausal osteoporosis there is a clear association between low bone mineral density and high risk of fracture; most studies in diabetics paradoxically report either similar bone mineral density or 5–10% increase in bone mineral density of patients with T2DM compared to nondiabetic controls (7). At a microstructural level, MRI and high-resolution peripheral quantitative computer tomography studies indicate increased cortical porosity,

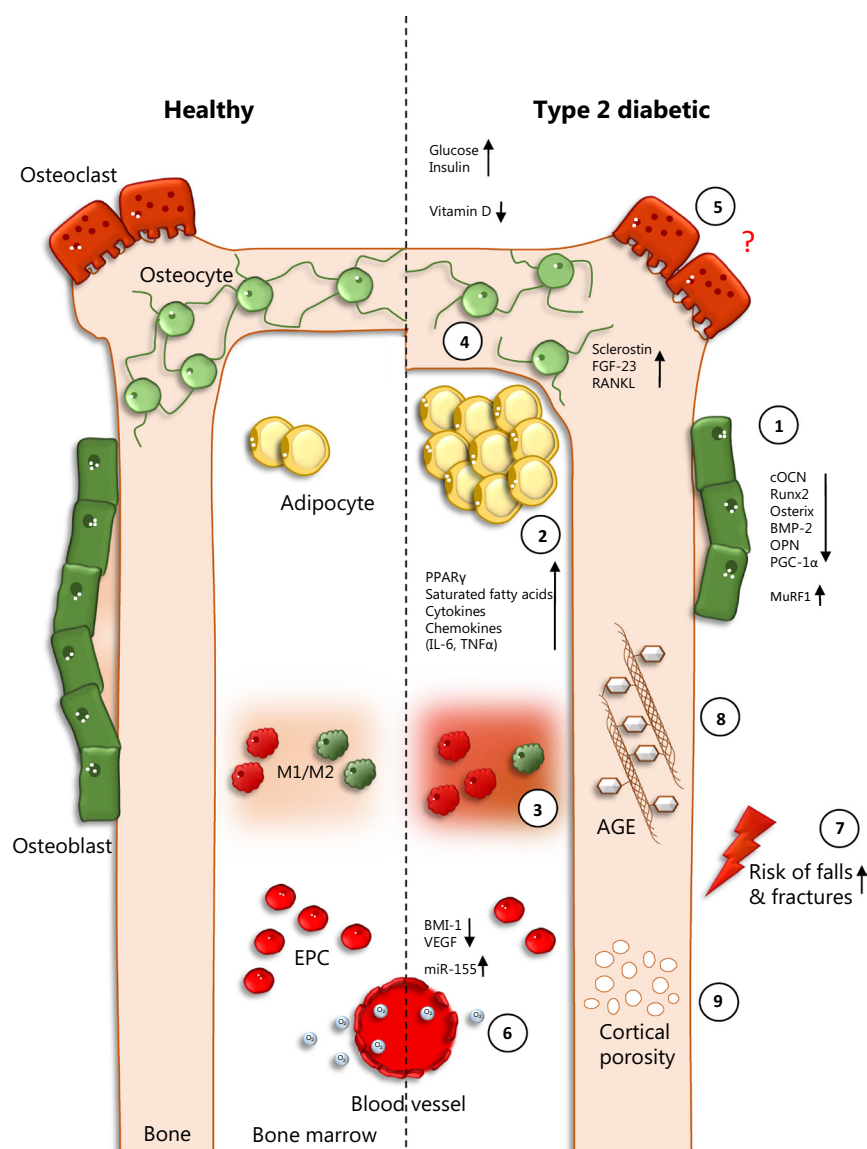


Figure 1 Effects of T2DM on bone homeostasis. T2DM negatively affects trabecular bone mass, while cortical bone mass is increased. (1) The number and function of bone-forming osteoblast is reduced. In addition, vitamin D serum levels are decreased, which alters calcium and phosphate homeostasis. (2) Osteoblasts derive from MSC that favor differentiating into fat-storing adipocytes in T2DM leading to bone marrow adiposity and increased expression of cytokines and chemokines as well as to an elevated amount of free unsaturated fatty acids. (3) This results in increased inflammation leading to accumulation of pro-inflammatory M1 macrophages and reduced switch into anti-inflammatory M2 macrophages. (4) The network of osteocytes is reduced due to an increased apoptosis rate. They increase their expression of sclerostin, an inhibitor of osteoblast function, and RANKL, a promoter of osteoclastogenesis. FGF-23, a phosphaturic hormone, is additionally increased. (5) Effects on osteoclasts are controversial in the literature, but T2DM is generally accepted to reduce bone turnover and thus also osteoclast function. (6) The amount of endothelial progenitor cells (EPC) is reduced in T2DM leading to vessel permeability. In addition, T2DM causes microhypoxia in bone niche, which in turn increases inflammation. (7) T2DM patients have an increased risk of falls and fractures due to reduced bone quality indicated by (8) an increased formation of advanced glycation end-products (AGEs) and (9) cortical porosity.

especially in patients with fractures and/or microvascular disease (23, 24, 25). Accordingly, bone strength estimated by finite element modeling at the distal radius is lower in patients with T2DM and correlates negatively with cortical porosity (26). Finally, microindentation on the tibia of patients with T2DM revealed low bone material strength (27, 28, 29). Taken together these studies provide evidence that the biomechanical integrity of the skeleton is reduced in T2DM, and this is a result of an inferior microstructure and material properties of the bone tissue. Interestingly, the trabecular bone score, a parameter that evaluates the pixel gray-level variations in lumbar spine dual-energy X-ray absorptiometry images and is related to bone microarchitecture independent of bone mineral density, appears to pick up those microarchitectural differences, being consistently low in patients with T2DM (30, 31, 32).

The effect of T2DM on bone material properties may not be limited to porosity, as alterations in bone collagen also occur. It is unclear to date, however, whether these significantly contribute to a weakened bone material in diabetic patients. One alteration of collagen that is highly researched is the formation of AGEs. AGEs are modifications of proteins that become nonenzymatically glycosylated after exposure to sugars (33), and are elevated in individuals with hyperglycemia. AGE cross-links alter the properties of proteins such as collagen and laminin (34, 35) and, in bone, this leads to an increased brittleness of the otherwise elastic collagen fibers (36, 37) and reduces the tissue toughness (38, 39). AGEs can also interfere with osteoblast (40, 41) and osteoclast functions (42), and may also impair osteocyte response (43, 44). AGEs may therefore play an important role in both bone material properties and bone turnover in T2DM (45, 46, 47).

In animal models of T2DM, some studies have reported increased AGE content in the bone tissue (48, 49), while other studies have reported no difference in AGEs but alterations in the collagen structure (50) and increased collagen maturity (51). Since levels of AGEs in the bone tissue cannot be measured noninvasively in patients, surrogates, such as serum or urine levels, are typically used. Due to the fluorescent nature of AGEs, skin fluorescence has also been reported as a surrogate measure and has been associated with the reduction of bone material strength assessed with indentation tests (29). To date, relatively few studies have investigated the accumulation of AGEs in T2DM patients, in particular, in the bone tissue and those that have report conflicting results. Serum levels of the AGE pentosidine in T2DM patients have been reported to be higher than (52) and similar to (27, 53, 54)

nondiabetic controls. A recent study that examined AGEs in the bone tissue from femoral neck specimens excised during total hip replacement surgery reported increasing trends of AGE levels in the cortex, but not trabecular bone from T2DM patients, despite no significant differences in serum levels of pentosidine or total AGEs between groups (27). Although the authors do report significant correlations between bone and serum AGEs in this study, these results highlight that surrogate measures may not be sufficient as diagnostic tools for assessing the material properties of T2DM bone. Nevertheless, pentosidine levels in the urine have also been associated with increased vertebral fracture prevalence in T2DM patients and lower trabecular bone score, but not in controls (53, 55), which along with the microstructural studies reporting increased porosity, suggests that a subset of T2DM patients may be at a particularly high risk of fracture.

Mechanisms of bone fragility in T2DM

In addition to the microstructural alterations in organic and inorganic bone components, T2DM directly affects the differentiation and function of bone cells, and modifies the bone microenvironment in such a way that secondary negative effects on bone cells occur (e.g. reduced blood flow in bone, increased presence of fat in the bone marrow, inflammation). The mechanisms of how T2DM exerts these negative effects on bone cells will be discussed in this article.

Direct effects on bone cells

Osteoblasts and bone formation parameters

Osteoblasts derive from mesenchymal stromal cells (MSC) and are essential for bone formation as they synthesize collagen and mineralize the organic matrix. T2DM individuals have high serum glucose concentrations, elevated post-load insulin levels and a high body mass index (56). In homeostasis, insulin promotes osteoblast differentiation leading to an increased expression of the carboxylated form of osteocalcin. Similarly, mouse models showed that glucose is an important source of energy for osteoblasts to allow for the production of collagen fibers (57). As previously reviewed, in T2DM, however, high levels of glucose suppress osteoblast differentiation (58). Also, carboxylated osteocalcin serum concentrations are reduced and inversely associated with fasting glucose levels and insulin resistance (reviewed in 59). In line, a reduced osteoid volume, osteoid thickness and osteoblast

surface was detected in iliac crest bone samples in male and female T2DM individuals (11). Furthermore, the serum bone formation markers procollagen type 1 N-terminal propeptide and alkaline phosphatase (ALP) have been found mostly unaltered or reduced in type 2 diabetic individuals, even though increased levels of ALP have also been reported (reviewed in 9, 60, 61, 62, 63, 64).

As mentioned above, high glucose concentrations lead to the creation of AGEs in bone matrix. Human osteoblasts treated with high glucose concentration and/or AGEs show a reduced expression of pro-osteogenic markers such as Runx2 and Osterix (65, 66). More critically, AGEs increase the rate of apoptosis of osteoblasts and its precursor cells (67, 68).

Wnt signaling and the bone morphogenetic pathway (BMP) are critical for osteoblast differentiation. Osteogenic cell lines show reduced Wnt activity, which is associated with reduced osteogenic differentiation, after stimulation with high glucose concentrations (69, 70, 71). Wnt signaling is also one of the key pathways that regulate the osteoblast vs adipocyte fate decision of MSCs. In T2DM, osteogenesis is reduced while adipogenesis is increased resulting in bone marrow adiposity due to increased PPAR γ signaling, which is at least partially Wnt-dependent (reviewed in 4, 72, 73, 74, 75). Other pathways determining osteoblast vs adipocyte fate in T2DM are discussed in section 'Indirect T2DM effects on bone cells'. Besides reduced Wnt signaling, a decreased expression of BMP-2 and osteopontin was also found in osteoblasts obtained from type 2 diabetic rats (76). In accordance with suppressed Wnt signaling *in vitro*, serum concentrations of the Wnt inhibitors, sclerostin and DKK-1, are increased in T2DM (12, 77) as are serum levels of active transforming growth factor β (TGF- β), which is associated with the development of diabetic nephropathy (78). Culturing pre-osteoblasts in this T2DM serum leads to reduced ALP activity and diminished matrix mineralization (66, 79).

Finally, the fatty acid composition has a large impact on osteoblast function. Nonesterified fatty acids induce apoptosis in osteoblasts, which is associated with downregulation of peroxisome proliferator-activated receptor γ coactivator 1- α (PGC-1 α) and upregulation of the muscle ring finger protein-1. Depletion of muscle ring finger protein-1 or upregulation of PGC-1 α in diabetic mice restored bone mass to WT level without affecting T2DM (80). Not only the amount but also the saturation of fatty acids affects osteoblast function. T2DM has an increased amount of saturated compared to monounsaturated fatty acids leading to reduced osteoblast differentiation and

mineralization capacity as well as increased apoptosis rate due to their lipotoxic effect (81).

Taken together, T2DM exerts direct negative effects on osteoblasts via several molecular mechanisms (Fig. 1). Moreover, it favors the fate decision of MSCs to turn into adipocytes, which further impairs osteoblast function, bone formation and bone mass.

Osteocytes

During bone formation, a proportion of osteoblasts embed themselves into the bone matrix they produced and differentiate into mechanosensing osteocytes. For the exchange of oxygen and nutrients, they are connected via canaliculi and form a sophisticated network through the bone (82). This network is impaired in T2DM and even under high-fat diet conditions (83). The osteocyte density is reduced and the number of empty lacunae is increased likely due to increased osteocyte apoptosis under high glucose conditions. This subsequently leads to an impaired mechanosensing response to oscillatory shear stress *in vitro* (44, 84). Osteocytes coordinate osteoblast and osteoclast function via secretion of the Wnt inhibitor sclerostin and the promoter of osteoclastogenesis, receptor activator of NF- κ B ligand (RANKL), respectively (85, 86). *In vitro*, high concentration of glucose and incubation with AGEs increase both sclerostin and RANKL expression (44). In T2DM patients, sclerostin serum levels are elevated and associated with glycated hemoglobin levels and insulin resistance (87, 88). Lastly, osteocytes regulate phosphate homeostasis by expression of fibroblast growth factor-23. This factor is also involved in the progression of atherosclerosis through its effects on endothelial cell function and is a predictor of cardiovascular disease risk (89, 90). Accordingly, fibroblast growth factor-23 serum concentrations are increased in T2DM patients that have high risk for developing cardiovascular diseases (91).

Osteoclasts

For a healthy bone status, bone formation by osteoblasts and bone resorption by osteoclasts needs to be balanced. In T2DM, osteoblast function is disturbed and osteoclast activity is altered leading to impaired bone remodeling. However, the literature on osteoclasts is controversial. Serum levels of the bone resorption marker collagen type I C-terminal telopeptide (CTX) are reported to have either increased or decreased in T2DM cohorts (reviewed in 60). A meta-analysis of 66 studies however revealed an overall

low bone turnover with low levels of CTX in diabetic patients (13). In type 2 diabetic rodents (TallyHo mice and ZDF rats), bone resorption parameters are mostly increased (serum CTX or TRAP, histological numbers of osteoclasts) (16, 17, 76, 92).

Culturing osteoclast-like Raw264.7 cells in high glucose concentration reduces the expression of osteoclast-specific genes including nuclear factor of activated T cells, cytoplasmic 1, tartrate-resistant acid phosphatase and osteoclast-associated receptor. In addition, high glucose decreases cell proliferation and cell size due to suppression of the formation of the osteoclast-specific actin ring (70). When mimicking hyperglycemia and hyperinsulinemia combined, osteoclast differentiation and gene expression of marker genes are downregulated (93).

For osteoclastogenesis to occur, RANKL must activate its receptor RANK located at the surface of pre-osteoclasts. Osteoprotegerin (OPG) acts as a decoy receptor of RANKL and thereby inhibits osteoclast differentiation. Both, RANKL and OPG are highly expressed by osteoblasts and osteocytes. Osteoblasts cultured in high glucose concentration increase both RANKL and OPG expression (70, 94) while the direct effect of RANKL on osteoclastogenesis is reduced (95). In addition, incubation of osteocyte-like MLO-Y4-A2 cells with high glucose concentration and AGEs highly increases RANKL expression (44).

Besides osteogenic cells, other cells contribute to RANKL and OPG production under certain, for example, inflammatory conditions. T2DM patients suffer from body and bone marrow adiposity (reviewed in 72) which is associated with increased TNF α serum level (96). Human bone marrow adipocytes express more RANKL and less OPG when additionally treated with TNF α resulting in an increased resorption capacity of osteoclasts (97). In addition, TNF α can induce osteoclastogenesis in combination with macrophage colony-stimulating factor only (98) and also potently increases osteoclastogenesis when low RANKL concentrations are present (99).

Finally, T2DM is associated with a higher amount of saturated fatty acids that reduce osteoclastogenesis, but increase osteoclast survival by production of macrophage inflammatory protein 1 α leading to activation of NF- κ B (100, 101). Taken together, several diabetes-derived factors have an impact on osteoclasts, yet, sometimes in opposing ways. Thus, current data do not allow forming a general statement on the role of T2DM on osteoclasts.

Indirect T2DM effects on bone cells

Bone marrow adiposity and MSC fate

Recently, bone marrow fat was discovered as an endocrine organ (72, 102). Even though it is known to impact energy homeostasis and bone turnover via secretion of adiponectin, still its origin, detailed characterization and function remain largely elusive. Studies in T2DM patients show controversial results. The vertebral bodies of type 2 diabetic men and postmenopausal women show a higher marrow adipose tissue (MAT) content compared to controls. MAT is negatively correlated with bone mineral density and positively with visceral adipose tissue and HbA1c values (74, 103). However, two other studies with T2DM patients showed no difference in MAT. Nonetheless, MAT was associated with HbA1c in one study (104) and with fractures in another (103, 105). High MAT mass is further associated with increased PPAR γ signaling and accordingly treatment of T2DM patients aged with the PPAR γ agonist pioglitazone increased MAT in vertebra and femur (73).

Similar to humans, bone marrow adiposity increases in rodents with T2DM. Among others, diabetic ZDF rats and TallyHo mice show an increased bone marrow fat mass (up to 50-fold) compared to nondiabetic controls in vertebra and long bone, while bone mass is decreased (17, 76, 106, 107). In addition, feeding C57BL/6 mice with high-fat diet over several months results in an accumulation of bone marrow adiposity and a reduced bone mass (108). Interestingly, control of blood glucose affects MAT. While fat volume in the vertebra is unaltered by insulin therapy in diabetic ZDF rats, the adipocyte area, but not the size of adipocytes in the tibia, is reduced (107).

Mechanistically, high glucose concentrations prime MSCs to reprogram autocrine Wnt signaling resulting in an increased *WNT11* expression and activation of PKC leading to an elevated adipogenesis (109). Further, Wnt5a plays an important role in MSC fate decision. Wnt5a-deficient mice express less LRP5/6 leading to a reduced Wnt/ β -catenin signaling, which consequently reduces osteoblastogenesis while increasing adipogenesis (110). Similar pro-osteogenic and anti-adipogenic effects were detected for the Wnt ligands Wnt6, Wnt10a and Wnt10b (111, 112). In line, blocking β -catenin signaling leads to bone marrow adiposity and low bone mass (113). Recently, other factors were identified to control MSC fate decision. The nuclear transcription factor I-C increases adipogenesis when being overexpressed and thereby reduces osteoblastogenesis and vice versa when its expression is

inhibited (114). In addition, the cell surface protein Thy-1 – also known as cluster of differentiation 90 – controls MSC differentiation by promoting osteoblastogenesis and decreasing whole body adipogenesis *in vivo* (115). In patients with osteoporosis and obesity, both characterized by altered bone homeostasis, serum concentrations of soluble THY-1 are reduced indicating clinical relevance of this factor (115). Therefore, bone marrow adipogenesis in T2DM must result from multifactorial reasons such as altered Wnt signaling, modified expression of adipokines, transcription factors and surface proteins as well as augmented glucose and insulin signaling (116).

Inflammation

Type 2 diabetic patients are overweight and adiposity gives rise to low-grade inflammation that negatively affects whole body metabolism and bone homeostasis (60). In T2DM patients, serum levels of pro-inflammatory cytokine interleukin 6 (IL-6) and high-sensitivity C-reactive protein are increased, which is associated with reduced concentration of osteocalcin (117). TNF α , IL-1 and TGF- β levels are also highly increased in overweight and insulin resistance indicating latent inflammation in T2DM (reviewed in 118, 119). Further, the amount of saturated fatty acids is increased (81). Stimulation of human osteoblasts with saturated fatty acids highly increases expression of IL-6 and the chemokines IL-8, and monocyte chemoattractant protein-1 (120). Finally, hypoxia is a novel mechanism participating in insulin resistance in adipose tissue of obese patients that exacerbates the pro-inflammatory activity of adipocytes (121, 122, 123).

Inflammation activates immune defense by mobilization of macrophages. Increased body and bone marrow fat in T2DM attract monocytes via elevated chemokine expression such as leukotriene B4, macrophage inflammatory proteins, macrophage migration inhibitory factor and monocyte-chemotactic protein 3. In fat depots, they differentiate into pro-inflammatory M1 macrophages and further express pro-inflammatory cytokines resulting in macrophage accumulation and activation of inflammatory reactions. This disturbs macrophage polarization leading to a reduced switch from pro-inflammatory M1 to anti-inflammatory M2 macrophages, which are important for tissue surveillance, remodeling functions and maintaining insulin sensitivity of white adipose tissue (reviewed in 124) (Fig. 1).

Microangiopathy in bone

A healthy status of vascularization is mandatory to provide all body cells with nutrients and oxygen. Also within the bone microenvironment, angiogenesis is important and in fact linked to osteogenesis (125). In diabetic mice, the blood flow and microvascular density in bone marrow is reduced and the amount of endothelial cells is decreased. They are functionally impaired as shown by a diminished capacity to migrate and to form networks, which leads to microangiopathy and increased vessel permeability (126, 127). RhoA-Rho-associated kinase signaling has been implicated in reduced vessel function as a result of reduced stem cell viability, mobilization and via elevated oxidative stress (128, 129). In line with that, T2DM patients have a reduced abundance of endothelial progenitor cells in the blood (130, 131, 132, 133). In human endothelial progenitor cells, levels of cell survival regulating microRNA miR-155 are increased resulting in elevated apoptosis, which is triggered by high glucose concentrations (132, 134). To mobilize endothelial progenitor cells from the bone marrow, nitric oxide synthase (eNOS) is necessary. Under diabetic conditions endothelial progenitor cells synthesize less nitric oxide due to a damaged eNOS-caveolin-1 complex (135, 136). This endothelial dysfunction is also associated with increased Dickkopf-1 serum levels that further negatively affect osteoblast differentiation (77).

In addition, T2DM alters adipokine expression. Adiponectin confers protection of endothelial cells and its serum concentration is reduced in diabetic individuals, which may lead to microangiopathy (reviewed in 137). *In vivo*, the decreased endothelial progenitor cell mobilization from bone marrow leads to less cell recruitment in ischemic tissue. Therefore, fewer endothelial progenitor cells participate in neovascularization in peripheral tissues, leading to organ dysfunction and impaired regeneration potential, such as seen in fracture healing in T2DM patients (138). These findings indicate that vascular dysfunction in diabetes has its origin in the bone marrow by depleting the stem cell niche.

Treatment of diabetic bone disease

Two strategies can be envisaged to treat diabetic bone disease. First, to control blood glucose levels and prevent secondary effects of T2DM, and second, by directly blocking bone resorption and restoring osteoblast

Table 1 Effect of antidiabetic and anti-osteoporosis treatments on bone.

Treatment	Animal <i>in vivo</i> studies				Human <i>in vivo</i> studies			
	Bone formation	Bone resorption	BMD	Fracture healing	Bone formation	Bone resorption	BMD	Fracture risk
Antidiabetic treatment								
Metformin	↑	↓	↑*	↑	↓/=	↓/=	↑/=	↓/=
Thiazolidinediones	↓	↑	↓/=	?	↓/=↑	↑/=↑	↓/=	↑/=
Insulin	↑	=	↑/=	↑	=	=	=	↑/=
Sulphonyl urea	↑	↓	↑	?	↑/=	↓/=	=	↑
Incretins	↓	↓	↑/=	?	↓/=	↓/=	↑/=	↓/=
SGLT2	=	↑/=	=	?	=	↑/=	=	↑/=
Anti-osteoporosis treatment								
Bisphosphonates	↓*	↓*	=*	↑* ¹	↓	↓	↑/=	↓
Anti-RANKL Ab	↓*	↓*	↑*	↑* ¹	↓* ¹	↓* ¹	↑* ¹	↓* ¹
Intermittent PTH	↑	↓	↑	↑	↑	↓	↑	↓
Anti-sclerostin Ab [#]	↑	↓	↑	↑	↑* ¹	↓* ¹	↑* ¹	↓* ¹

*Only tested in type 1 diabetes; *¹only tested in nondiabetics; #not yet approved.

↓, decreased; ↑, increased; ?, not investigated; =, unaltered; BMD, bone mineral density; PTH, parathyroid hormone; SGLT2, sodium-glucose cotransporter 2.

function. Both options will be discussed in this article (Table 1).

Effects of antidiabetic treatments on bone

Metformin

Metformin is taken orally and decreases production of hepatic glucose via inhibition of mitochondrial respiratory chain complex 1, while increasing insulin sensitivity and stimulating glucose uptake by activation of AMP-activated protein kinase ((139), reviewed in 140). *In vitro* and *in vivo*, metformin increases osteogenic induction and further enhances bone repair in rodents. In T2DM patients, metformin does not alter bone mineral density or fracture risk at spine, forearm or hip (reviewed in 6, 140, 141, 142). Recently, it was shown that metformin therapy for 18 months does not alter spinal or hip bone mass, while trabecular bone score decreases in T2DM patients. Further, serum bone turnover markers P1NP and CTX are decreased by metformin (30, 143).

Thiazolidinediones

Thiazolidinediones (TZD) improve glycemic control and insulin sensitivity in T2DM. A common agent is rosiglitazone that activates PPAR γ and improves insulin sensitivity in adipocytes by promoting adipogenesis and the accumulation of triglycerides into lipid droplets, thereby increasing adipose tissue amount (140). *In vivo*, TZD administration leads to bone marrow adiposity and reduces osteoblastogenesis resulting in bone loss. In line, T2DM patients with TZD treatment have increased bone marrow fat in the fourth lumbar vertebral body, reduced

osteoblastogenesis and an increased fracture risk at the hip and wrist, which is generally more prominent in women (73, 144, reviewed in 140, 143). It is interesting to note that TZD treatment is not associated with risk of fracture in men (meta-analysis). However, recently it was detected that rosiglitazone administration is not associated with alterations in bone turnover markers, while reducing bone formation in combination with metformin (143).

Insulin

T2DM is characterized by increased serum glucose and insulin concentration due to insulin resistance. With disease progression, insulin production decreases and patients need to be additionally treated with insulin. Daily insulin treatment of T2DM rats improved glycemic control, but did not improve trabecular bone mass while cortical bone mass increased, suggesting site-specific effects of insulin on bone. Additionally, bone defect regeneration improved up to control level after insulin administration (107). Human studies are lacking to evaluate the effect of insulin treatment on bone mass in T2DM. Nevertheless, studies show that T2DM patients treated with insulin have an unaltered to increased fracture risk at the hip, while men seem to be more prone than women (reviewed in 140, 145, 146). It is clear that insulin treatment has a positive effect on bone metabolism. Thus, the increased fracture risk may be explained by the higher rate of falls caused by hypoglycemic events. It is also worth to note that patients on insulin treatment may also have longer duration of disease and are more likely to have developed diabetes complications.

Sulphonyl urea

These medications have been used for more than 40 years, are inexpensive and still largely used in the diabetes clinic. Mechanism of action is based on increased release of insulin from the beta cell that is independent from glucose serum levels, implying a high risk of hypoglycemic events. Preclinical studies have excluded a direct effect of sulphonylureas at the bone level and, therefore, have been considered a safe option. However, more recent data have shown almost a doubled risk of hip fracture in treated patients, likely caused by higher frequency of hypoglycemic (146, 147, 148) events.

Incretins

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are two gastrointestinal hormones known as incretins, 'INtestine SeCRETion Insulin', secreted in response to nutrient intake. In order to compensate for low incretin effect in T2DM patients, two different therapeutic options have been developed, either by inhibiting dipeptidyl dipeptidase-4, an enzyme that rapidly inactivates GIP and GLP-1 (sitagliptin, vildagliptin, saxagliptin, linagliptin, alogliptin), or by GLP-1 mimetic drugs (liraglutide, exenatide, dulaglutide, albiglutide, lixisenatide). These medications improve glucose control with low risk of hypoglycemic events and can be safely used in the long term.

GLP-1 receptor knock-out mice show increased osteoclast numbers, bone resorption rate, bone fragility and low calcitonin levels, which increases after treatment with a GLP-1 receptor agonist (149). Treatment with GLP-1 analogues under normal or high glucose conditions in ovariectomized rats and mice for 16 weeks promoted bone formation and lowered bone resorption with a significant increase in femoral bone mineral density and strength (150, 151, 152, 153, 154). In T2DM rodents, GLP-1 and exendin-4 treatment reduced serum bone remodeling marker while increasing bone mass only partially (155, 156, 157). Despite these promising preclinical results, clinical data have only shown a neutral effect on bone mineral density and no prospective data are available for fracture risk. In an attempt to fill this gap, different meta-analyses on randomized controlled trials, where fractures were noted as possible side effects, have been published with inconsistent results (158, 159, 160, 161). These studies are limited by different fracture definitions across studies and lack of radiographic control of the events. Women treated with liraglutide for 52 weeks lost 12% of initial body weight, had improved total, pelvic and

arm/leg bone mineral content and P1NP levels, although methodological concerns have been raised (162). Risk of fracture has been shown to be neutral (HR 1.00 (95% CI 0.83–1.19)) in a large clinical trial investigating the cardiovascular safety of saxagliptin vs placebo (163). In conclusion, although more clinical evidence is needed, GLP-1 agonists and dipeptidyl dipeptidase-4 inhibitors seem to have a safe bone health profile.

Sodium-glucose cotransporter inhibitors

These new generation drugs selectively inhibit the renal sodium-glucose cotransporter 2 (SGLT2, the key transporter mediating glucose reabsorption by the kidney), thereby increasing urinary glucose excretion (164, 165). The first three SGLT2 inhibitors released in the market, namely canagliflozin, empagliflozin and dapagliflozin, have proven not only an antidiabetic effect but also significant reduction in cardiovascular risk, implying a wide use in both T1DM and T2DM. While *in vivo* and *in vitro* studies have not been consistent, clinical data have raised concerns on bone safety (4, 166). In fact, SGLT2 inhibitors increase phosphate tubular reabsorption and serum concentrations of parathyroid hormone and, in turn, bone (167). In two different randomized, double-blind, placebo-controlled studies, dapagliflozin has shown neutral effects on bone turnover and bone density (168, 169). A *post hoc* analysis from the EMPAREG study has also shown neutral effects on fracture risk for empagliflozin (170).

Data are less reassuring for canagliflozin which has been associated with higher bone loss, increased bone resorption and a higher incidence of fractures compared to non-canagliflozin treatment (2.7 vs 1.9% respectively) (171, 172). Although data on dapagliflozin and empagliflozin show a safe bone profile, data on canagliflozin are concerning and more studies are needed to clarify a possible class effect and reasons for discrepancy in terms of safety profile among these medications.

Anti-osteoporosis treatments

Bisphosphonates

Bisphosphonates are the first-line option to treat osteoporosis in postmenopausal women and men. Bisphosphonates bind to mineralized matrix with a high affinity and inhibit the resorption capacity of osteoclasts. This reduces bone turnover, increases bone mass and decreases fracture risk (reviewed in 173). Elderly postmenopausal, osteoporotic women with T2DM that

are treated with alendronate and calcium/vitamin D supplements are resistant against therapy effects shown by decreased total hip, femur neck and femur bone mineral density, while spine bone mineral density is unaltered (174). In contrast, when osteoporosis had been diagnosed recently, T2DM women benefited from bisphosphonate treatment by increased bone mineral density at the lumbar spine. However, bone remodeling markers such as CTX and ALP are decreased in T2DM, osteoporotic women with increased spine bone mineral density (reviewed in 175). In addition, treatment with bisphosphonates has a similar anti-fracture efficacy in diabetes patients compared with nondiabetic regarding non-vertebral fractures (reviewed in 176). Thus, even though bisphosphonate further suppress bone turnover, they may still be an effective therapy for patients with T2DM.

Denosumab

Denosumab is a monoclonal antibody targeted against RANKL, the key cytokine to drive osteoclastogenesis, which has been approved for the treatment of osteoporosis. Through its mechanism of action, inhibition of RANKL reduced bone resorption and therefore increased bone mass in rodents (177). In addition, the blockade of RANKL has been tested in preclinical models to improve glycemic status. A *post hoc* analysis of the freedom trial has shown that denosumab significantly lowers fasting serum glucose in naive patients with T2DM throughout the 3 years of observation vs placebo (178). Considering that the study was not designed for diabetes-related outcomes, more studies are needed to test efficacy of denosumab on glucose control. Nonetheless, phase II studies are ongoing to better define denosumab as a safe option to treat bone fragility in type 2 diabetic patients.

Intermittent PTH treatment

Intermittent PTH administration is commonly used in the clinics to treat osteoporosis by enhancing bone remodeling, especially osteoblast function, resulting in enhanced trabecular bone parameters (179). In T2DM rats, intermittent parathyroid hormone treatment increases trabecular bone mass due to elevated bone formation and enhances bone defect regeneration (14).

Post hoc analyses from the dance study have shown that teriparatide decreases risk of fracture in diabetics similar to nondiabetic patients (180). However, the results on bone mineral density were surprising, showing a significantly greater effect of teriparatide at the femoral

neck in diabetics vs nondiabetics. These data are supported by a recent study from Langdahl *et al.* (181), who found that teriparatide reduces vertebral, non-vertebral and femur fractures in diabetics. Given the mechanism of action, teriparatide should be the first-line treatment in diabetic patients after a fragility fracture. Improving bone formation but also gradually increasing bone resorption appears as an ideal order to reverse the low bone turnover state typical of long-term diabetes.

Anti-sclerostin antibody

Sclerostin is a negative regulator of bone mass by inhibiting osteoblastogenesis. *In vivo* studies show that treatment with anti-sclerostin antibody improves bone mass in healthy rodents and monkeys as well as in type 2 diabetic rats (reviewed in 182, 183). In diabetic ZDF rats, bone formation rate increases leading to an elevated bone mineral density and enhanced bone mechanical properties. In addition, bone defect healing was improved by anti-sclerostin antibody administration (15). In clinical trials, the anti-sclerostin antibody romosozumab increased bone mineral density and reduced fracture risk in postmenopausal women by increasing bone formation and transiently decreasing bone resorption. Therefore, this treatment option may be a good candidate for the treatment of diabetic bone disease. However, romosozumab treatment has been associated with an increased risk of cardiovascular events, which are common in T2DM patients, and therefore romosozumab needs to be critically tested for clinical use for T2DM (reviewed in 183, 184).

Conclusion

Research over the past years has highlighted the detrimental effect of T2DM on bone quality and strength and has led to the acceptance of diabetic bone disease as a serious complication of long-standing T2DM. Despite recent progress in the understanding of the pathogenesis of diabetic bone disease, the mechanisms of action are far from being completely understood. Moreover, with the emergence of novel antidiabetic treatments, prospective studies are required to evaluate their effects on bone health and identify which treatments may require co-treatment with anti-osteoporosis medications. Thus, in light of the increasing prevalence of T2DM, more basic and clinical insights are required to maintain bone health of type 2 diabetics.

Declaration of interest

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