【 REVIEW ARTICLE 】

Diabetes Mellitus-induced Bone Fragility

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Abstract:

Accumulating evidence has shown that the risk of osteoporotic fractures is increased in patients with diabetes mellitus (DM). Thus, DM-induced bone fragility has been recently recognized as a diabetic complication. Because the fracture risk is independent of the reduction in bone mineral density, deterioration of the bone quality may be the main cause of bone fragility. Although its mechanism remains poorly understood, accumulated collagen cross-links of advanced glycation end-products (AGEs) and dysfunctions of osteoblast and osteocyte may be involved. Previous studies have suggested that various diabetes-related factors, such as chronic hyperglycemia, insulin, insulin-like growth factor-I, AGEs, and homocysteine, are associated with the risk of bone fragility caused by impaired bone formation and bone remodeling. Furthermore, several antidiabetic drugs are known to affect bone metabolism and fracture risk. We herein review the association between DM and fracture risk as well as the mechanism of DM-induced bone fragility based on recent evidence.

Key words: diabetes mellitus, bone fragility, osteoporosis, advanced glycation end products, osteoblast, osteocyte

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Introduction

The population is aging rapidly worldwide; thus, assisting elderly individuals in maintaining self-control in terms of their activities of daily living (ADL) and quality of life (QOL) is an urgent task. The incidence of osteoporosis and type 2 diabetes mellitus (T2DM) is known to increase with aging. However, both diseases have been traditionally viewed as separate entities. Osteoporosis causes fractures in elderly individuals even with slight external force, subsequently worsening their ADL and QOL dramatically. In addition, their vital prognosis has been shown to also worsen after osteoporotic fractures, such as hip and vertebral fractures (1, 2). In contrast, recent studies have shown that patients with type 1 DM (T1DM) and T2DM have an increased risk of osteoporotic fractures (3-5). Therefore, DM-induced bone fragility has recently been considered a diabetic complication.

We recently showed that vertebral fractures are independently associated with all-cause mortality in patients with T2 DM (6). Furthermore, we conducted a survey by administering ADL and QOL questionnaires to patients with T2DM and found that osteoporosis and severe vertebral fracture were significantly associated with a decreased ADL and QOL even after adjusting for patients' background and other diabetic complications (7). The goal of diabetes treatment is to maintain the ADL and QOL of patients and to make their life spans similar to those of healthy individuals; therefore, developing a solution to reduce the risk of fractures in patients with DM is important.

In this article, the mechanism of DM-induced bone fragility and the clinical approach to its management are described.

Risk of Fractures in Patients with DM

Bone strength is defined as the sum of the bone mass and bone quality (8). Traditionally, osteoporosis has been thought to be a disease that reduces the bone mineral density (BMD) of patients. However, as some patients continue to experience fragility fractures even without a loss of BMD, the deterioration of bone quality is an important factor that causes weakened bone strength.
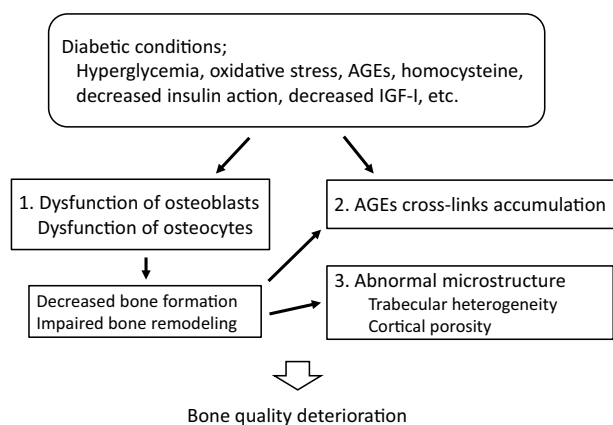
Figure 1. **The pathophysiology of DM-induced bone fragility. Many factors, such as hyperglycemia, oxidative stress, advanced glycation end products, homocysteine, and decreased insulin and insulin-like growth factor-I (IGF-I) activities, are involved in the pathophysiology of bone quality deterioration, which causes DM-induced bone fragility. 1) These factors affect bone-forming cells, particularly osteoblasts and osteocytes, resulting in decreased bone formation and impaired bone remodeling. 2) Chronic hyperglycemia and oxidative stress promote AGE collagen cross-links in the bone matrix. Impaired bone remodeling may contribute to the accumulation of AGE cross-links and 3) abnormal microstructure of trabecular and cortical bones. AGE: advanced glycation end-product**

Accumulating evidence has shown that the risk of osteoporotic fractures is significantly increased in both T1DM and T2DM patients. A previous meta-analysis showed that patients with T1DM slightly had a decreased BMD at the lumbar spine and hip (z-score, -0.22 and -0.37, respectively) while those with T2DM had a higher BMD at the lumbar spine and hip (z-score +0.41 and +0.27, respectively) (3). Based on the BMD values, the estimated fracture risks were increased 1.42-fold in T1DM and decreased 0.77-fold in T2 DM. However, the risks of hip fracture compared with non-diabetic controls were increased 6.94- and 1.4-fold in patients with T1DM and T2DM, respectively (3). Another meta-analysis showed that the hip fracture risk of patients with T1DM and T2DM were up to 6.3- and 1.7-fold higher, respectively, than that of non-diabetic controls (4). Furthermore, the risk of vertebral fracture in patients with diabetes has been shown to be up to 2.03-fold higher than in non-diabetic controls (9).

Based on a report on the integral analysis of three large-scale prospective studies, the BMD at the femoral neck compared with hip fracture risk in women and men with diabetes was 0.59 and 0.38 standard deviations (SD) higher than that in non-diabetic controls, respectively (10). We previously showed that the presence of T2DM was an independent risk factor for vertebral fracture in Japanese men and women after adjusting for age, body mass index, and lumbar BMD (odds ratio, 4.7 for men and 1.9 for women) (5). Taken together, these findings suggest that the increased risk of fracture is mainly caused by the deterioration of the bone quality but not a BMD reduction, and if we solely focus on BMD values, fracture risks in patients with DM might be underestimated.

Mechanism Underlying Bone Quality Deterioration in DM-induced Bone Fragility

With regard to the mechanism underlying the bone quality deterioration, 1) the accumulation of advanced glycation end-products (AGEs) in the bone matrix, 2) low bone formation and bone remodeling rates, and 3) abnormal bone microstructure are considered important (Fig. 1).

The bone matrix includes abundant type 1 collagens, and bones are able to maintain their flexibility and strength by forming physiological cross-links between collagen fibers. AGEs are generated by sequential nonenzymatic chemical glycoxidation of protein amino groups. AGEs are formed non-physiologically when patients have diabetes. Indeed, several studies have showed that the serum AGE levels in patients with diabetes were significantly higher than those in healthy subjects (11). AGEs are known to accumulate in various tissues, including the bone, kidney, brain, and coronary artery atherosclerotic plaques, with age.

Among AGEs, pentosidine is a well-characterized compound and is considered a good predictor for the development of micro- and macro-vascular complications in patients with diabetes (12). Saito et al. previously showed that spontaneous diabetic rats displayed a significant increase in pentosidine collagen cross-links in the bone, which was linked to a reduced flexibility of collagens and impaired mechanical properties despite a normal bone mass (13). Furthermore, a recent clinical study using bone biopsy samples in patients with T1DM showed that the pentosidine content in the trabecula was significantly and positively associated with the HbA1c levels and that it increased in patients with T1 DM and fracture (14). Because circulating pentosidine levels are correlated with pentosidine in the cortical bone, the serum and urine pentosidine levels may be useful as surrogate markers for its content in the bone as well as the bone strength. Schwartz et al. showed that higher urine pentosidine levels were significantly associated with increased clinical fracture incidence in elderly patients with T2DM in an observational cohort study (15). We also reported a cross-sectional study showing that serum pentosidine levels were significantly and positively associated with prevalent vertebral fracture in postmenopausal women with T2DM (16). Therefore, the accumulation of pentosidine collagen cross-links in the bone may be a major cause of BMD-independent bone fragility in patients with DM.

The suppression of bone formation and bone remodeling is also considered to be involved in bone fragility of patients with diabetes. Several clinical studies and meta-analyses have shown that bone formation markers, particularly serum osteocalcin, are significantly decreased in patients with both T1DM and T2DM compared with those in subjects without diabetes (17-20). In addition, bone histology data in patients

with T1DM and T2DM have indicated a markedly depressed bone formation rate (21-24). Previous studies using streptozotocin-induced and spontaneously diabetic rodent models showed that diabetes induces bone loss and impaired bone strength with a decreased bone formation rate (25, 26). Furthermore, serum osteocalcin levels were reported to be increased after intensive glycemic control in T2DM, whereas bone-specific alkaline phosphatase (BAP) level was decreased (27, 28).

In addition, the osteocalcin/BAP ratio was significantly associated with prevalent vertebral fracture in patients with T2DM (29). Osteocalcin is expressed in mature osteoblasts, and BAP is expressed in the early stage of differentiated osteoblasts; therefore, derangement of osteoblast maturation may be involved in the risk of fracture in patients with diabetes. In contrast, no definite opinion regarding bone resorption or osteoclast activity in diabetes has been shown thus far. Although several studies have reported that the levels of bone resorption markers, such as C-terminal telopeptide cross-links (CTX) and tartrate-resistant acid phosphatase (TRAP), deoxypyridinoline, and N-telopeptide of type I collagen (NTX), are comparable or higher in diabetic patients than in controls (30, 31), recent meta-analyses have shown a significant decrease in the levels of bone resorption markers, such as CTX and TRAP, compared with patients without diabetes (17). Given that the extent of BMD reduction in patients with diabetes is not high, it is difficult to conclude that bone resorption is remarkably increasing. Bone resorption is therefore assumed to be slightly but relatively increasing compared with decreased bone formation.

Indeed, a clinical study showed that lower levels of bone formation markers, such as P1NP, were associated with three- and two-fold increases in vertebral and overall fracture rates, respectively, in non-obese patients with T2DM compared with the rates in those without diabetes (32). In that study, patients with T2DM had lower levels of bone turnover markers, such as CTX, than patients without diabetes and those with impaired fasting glucose. In non-obese T2DM patients with high CTX levels, the increase in vertebral fracture failed to reach statistical significance compared with subjects without diabetes with high CTX levels, suggesting that low bone formation and turnover in patients with T2DM might be associated with an increased fracture risk.

In contrast, a higher body mass index (BMI) is traditionally considered to reduce the fracture risk due to increasing the BMD by suppressing bone turnover. However, recent studies have suggested that obesity may be a risk factor for fracture when adjusted for BMD (33, 34). We previously showed that the BMI was positively correlated with the BMD at the lumbar and femoral neck and negatively correlated with serum osteocalcin and urinary NTX levels in patients with T2DM, and that the patients with a high BMI and high HbA1c showed a significantly higher risk of vertebral fracture despite having a higher BMD than other groups (35). Therefore, obesity may contribute to decreased

bone turnover and be a risk factor of fragility fracture in diabetic patients, although further studies using diabetic subjects are needed to clarify this issue.

Bone tissue is constantly renewed by a balance between osteoblastic bone formation and osteoclastic bone resorption. Thus, when the bone remodeling process is disturbed, old bone tissue, including AGE collagen cross-links and microcracks, will not be renewed, leading to the deterioration of the bone quality. Osteocytes account for 90-95% of bone cells, and recent studies have shown that osteocytes play multifunctional roles in orchestrating bone remodeling by regulating both osteoblast and osteoclast functions. Sclerostin is specifically produced by osteocytes and inhibits osteoblast differentiation and bone formation by antagonizing the canonical Wnt-signaling pathway by binding to low-density lipoprotein receptor-related protein 5/6 receptors (36). Elevated serum sclerostin levels were shown to be associated with prevalent vertebral fractures in patients with T2DM independently of BMD and bone turnover markers (37, 38). These findings suggest that osteocyte dysfunction may contribute to bone fragility in patients with diabetes.

High-resolution peripheral quantitative computed tomography (HR-pQCT) is a three-dimensional imaging technology that evaluates the volumetric bone density, microarchitecture, and geometry separately for cortical and trabecular compartments. Thus, HR-pQCT may provide additional insight into the mechanisms underlying bone fragility. Based on a clinical examination using HR-pQCT, cortical bone porosity is considered to be an important factor influencing bone strength regardless of the BMD (39). Some studies have shown that T2DM is associated with deficits in the cortical bone (40-44), although other studies did not obtain the same results (45-47). In small case-control studies, patients with T2DM with prior fracture had a higher pore volume and cortical porosity at the radius than those without prior fracture (40, 41). Furthermore, a relatively large-scale study examining 129 patients with T2DM and 940 individuals without diabetes recently showed that T2DM was associated with a lower cortical volumetric BMD, higher cortical porosity, and smaller cross-sectional area at the tibia but not the radius (44). These findings suggest that the deterioration of the cortical microarchitecture, including cortical porosity, may be involved in DM-induced bone fragility, although further prospective studies are needed to confirm these suspicions.

The trabecular bone score (TBS) is an index to measure the distribution of cancellous bone microstructure. It can be used to estimate fracture risks regardless of BMD. In TBS measurement, images obtained from dual-energy X-ray absorptiometry are used for re-analyses, which means additional invasion is not necessary. In this sense, this measurement is expected to be used in the clinical setting in the near future. A previous study examining 57 and 43 women with and without T2DM, respectively, showed that the TBS in patients with T2DM was significantly lower than that in

those without diabetes, whereas the BMD was higher. Within the T2DM group, the TBS in women with good glycemic control (HbA1c ≤7.5%) was significantly higher than that in those with poor control (HbA1c >7.5%) (48). Furthermore, in a large-scale cohort study examining 1683 Japanese men, a decreased TBS was significantly associated with increased blood glucose levels and insulin resistance in patients with diabetes (49). Therefore, a linkage between diabetes-related osteoporosis and abnormal cancellous bone microstructures may be also possible.

Although the mechanism triggering bone microstructure abnormalities in diabetes has yet to be clarified, abnormalities of osteoblast and osteocyte functions have been suggested to be involved in impaired bone formation and bone remodeling, leading to bone microstructure abnormalities. Furthermore, osteocyte apoptosis may be associated with cortical porosity in DM-induced bone fragility.

Dysfunction of Osteoblasts and Osteocytes in DM

1. AGEs

Previous studies have shown that AGEs are involved in DM-induced bone fragility. Because AGEs have a physiological activity, they not only form collagen cross-links but may also directly impact bone-forming cells through the receptor for AGEs (RAGE). RAGE has been shown to be expressed in osteoblasts and osteocytes (50, 51), and hyperglycemia increases the RAGE expression (52). We previously showed that the combination of high glucose and AGEs inhibited the mineralization of osteoblastic MC3T3-E1 cells (52) and that AGEs inhibited the osteoblastic differentiation or mineralization of mouse stromal ST2 cells and human mesenchymal stem cells by decreasing the osterix expression, increasing the transforming growth factor (TGF)-β expression, and suppressing endoplasmic reticulum stress proteins (53-55). Furthermore, primary cultured human osteoblast-like cells (hOBs) from patients with T2DM and hip fractures showed slower proliferation and a lower expression of Runx2 and osterix when treated with high glucose or high glucose plus AGEs. Furthermore, high glucose induced a marked decrease in the receptor activator of nuclear factor κB ligand (RANKL)/osteoprotegerin (OPG) ratio in hOBs from patients with T2DM and hip fractures compared with those with hip fractures without DM (56). In addition, peripheral blood-derived mesenchymal stem cells isolated from patients with T2DM showed a significant decrease in the potential for differentiation toward osteoblasts, with lower levels of alkaline phosphatase (ALP), type 1 collagen, and osteocalcin as well as decreased mineralization compared with subjects without DM (57). The serum pentosidine and soluble RAGE levels measured by ELISA kits showed positive correlations with HbA1c, and RAGE expression was increased in the peripheral blood-derived mesenchymal stem cells of patients with T2DM compared with

those of subjects without DM.

We previously showed that high glucose and AGEs significantly increased the sclerostin expression in osteocyte-like MLO-Y4 cells (51). In contrast, AGEs reduced the RANKL expression, which stimulates osteoclast differentiation and activity. Furthermore, AGEs induced apoptosis of osteoblasts and osteocytes (51, 53). Taken together, these findings suggest that hyperglycemia and AGEs coordinately inhibit osteoblastic differentiation and bone formation directly and indirectly by increasing the sclerostin expression in osteocytes as well as contribute to a low bone rate of remodeling by decreasing the RANKL/OPG balance in osteoblasts and osteocytes (Fig. 2).

2. Homocysteine

Homocysteine (Hcy) is a sulfur-containing amino acid formed by the demethylation of methionine, and high plasma Hcy levels are caused by aging, genetic factors, lifestyle-related diseases such as diabetes and chronic kidney disease, and vitamin B6, B12, and folate insufficiencies. Excessive Hcy is known to induce endothelium dysfunction by increasing oxidative stress, resulting in a risk of cardiovascular diseases (58). Previous studies have shown that hyperhomocysteinemia increases the risk of osteoporotic fracture independently of BMD (59, 60), suggesting that the deterioration of the bone quality may be a dominant cause of Hcy-induced bone fragility.

With regard to diabetes, the blood Hcy concentration was thought to increase under conditions of insulin resistance (61, 62), which induces vitamin B consumption due to accelerated gluconeogenesis in the liver. Li et al. previously showed that plasma Hcy levels in patients with T2DM were significantly increased compared with those in subjects without diabetes, and that higher plasma Hcy levels were independently associated with osteoporotic fractures in patients with T2DM (63). Although the mechanism underlying the Hcy-induced bone fragility in DM remains unclear, several studies on the effects of Hcy on the osteoblast function and collagen cross-links have been reported (64, 65). Hcy reduces the formation of pyridinoline and deoxypyridinoline cross-links and decreases proteoglycan content, thereby directly altering the matrix organization, and negatively affects mineralization (66, 67), suggesting that Hcy causes decreased rates of matrix maturation and bone mineralization.

Saito et al. showed that diet-induced hyperhomocysteinemia decreased enzymatic cross-links and increased pentosidine cross-links although BMD was not affected (65), indicating that hyperhomocysteinemia induces the deterioration of the bone quality. Furthermore, Hcy directly affects osteoblast lineage cells, such as bone marrow stromal cells and osteoblasts. We recently showed that Hcy induced the apoptosis of osteoblastic MC3T3-E1 cells by increasing oxidative stress (64). In addition, Hcy suppressed the expression of the collagen cross-linker lysyl oxidase and increased the accumulation of extracellular pentosidine in osteoblasts. These findings suggest that Hcy may impair the viability
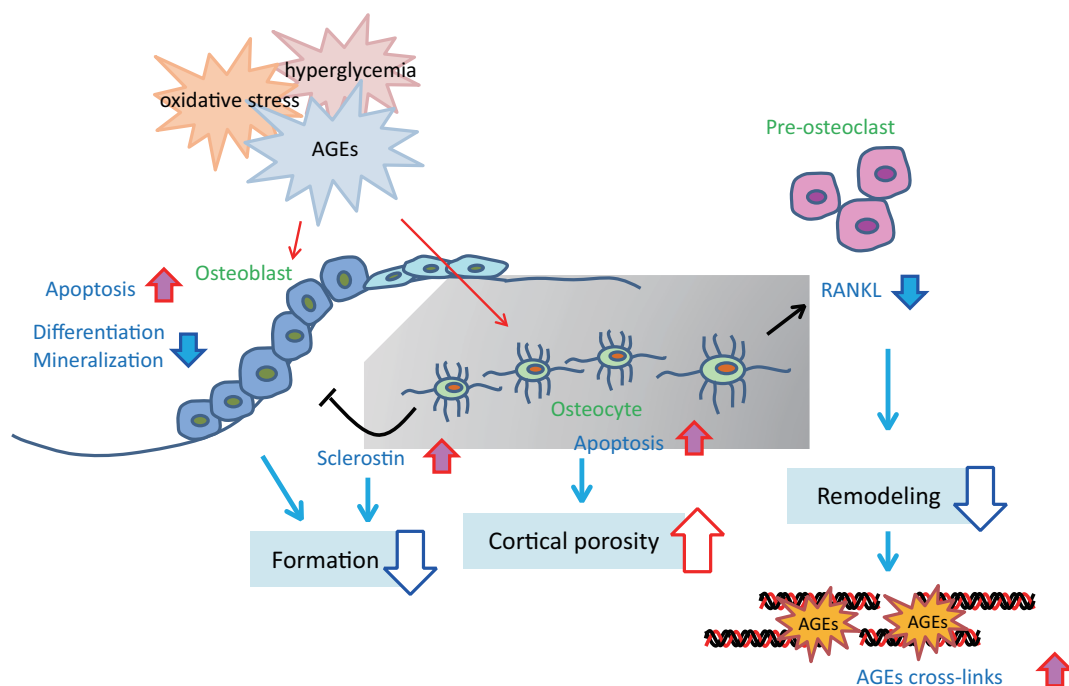
Figure 2. The mechanism underlying the diabetes-related bone fragility induced by AGEs. AGE formation is promoted by hyperglycemia and oxidative stress. AGEs induce apoptosis of osteoblasts and inhibit osteoblastic differentiation, resulting in decreased bone formation. AGEs induce apoptosis of osteocytes and enhance the sclerostin expression and decrease the RANKL expression in osteocytes, leading to the inhibition of the differentiation of osteoblasts and osteoclasts as well as a decreased rate of bone turnover and remodeling. The AGE-induced suppression of bone remodeling causes the accumulation of AGE collagen cross-links, resulting in the deterioration of the bone quality. Apoptosis of osteocytes induced by AGEs may be partly associated with cortical porosity. AGE: advanced glycation end-product, RANKL: receptor activator of nuclear factor κB ligand

and function of osteoblasts as well as exacerbate bone stiffness by inhibiting the formation of enzymatic collagen cross-links and increasing extracellular non-enzymatic pentosidine cross-links (Fig. 3). We also previously showed that Hcy increased oxidative stress and induced apoptosis of osteocytes by increasing the expression of NADPH oxidase 1 (Nox1) and Nox2 (68, 69). These findings suggest that Hcy induces osteoblast dysfunction and AGE accumulation and increases the apoptosis of osteocytes, resulting in decreased bone formation and bone remodeling and increasing AGE collagen cross-links.

3. Insulin

Insulin and its signaling pathway are important in osteoblastic differentiation, collagen synthesis, and bone formation. Patients with T1DM have a significantly reduced BMD with decreased bone formation and an increased risk of fragility fractures (70-72). These clinical features suggest that insulin signal is required for bone formation and bone development. Previous studies have shown that osteoblasts have a functional insulin receptor and that insulin treatment stimulates the proliferation and differentiation of osteoblasts (73, 74). Furthermore, osteoblast-specific insulin receptor knockout (*Ob-IR$^{-/-}$*) mice showed a reduced bone volume due to decreased bone formation and deficient number of osteoblasts (75, 76). The ALP activity and osteocalcin expression were significantly decreased by increasing the expression of a Runx2 inhibitor Twist2 in osteoblasts from *Ob-IR$^{-/-}$* mice (76).

In addition, insulin signaling in osteoblasts plays an important role in bone remodeling. Ferron et al. showed that insulin reduced the OPG expression in osteoblasts via FoxO 1 phosphorylation, leading to osteoblastogenesis and bone resorption (75). Although whether or not insulin resistance exists in bone remains unclear, Wei et al. showed in an *in vivo* study that high-fat diet (HFD)-fed mice had obesity and glucose intolerance as well as insulin resistance not only in the liver, muscle, and fat, but also in the bone (77). The mice showed decreased bone formation and bone turnover via increases in the OPG expression compared with normal diet-fed mice, although the bone volume was slightly increased in HFD-fed mice. With regard to the insulin function in the bone, these findings are consistent with those of previous studies (75, 76), although HFD-induced weight gain might help maintain the bone mass.

Tonks et al. recently showed that insulin resistance as assessed by hyperinsulinemic-euglycemic clamp is associated with a low bone turnover rate in humans (78). Subjects with obesity and insulin resistance and diabetic subjects had lower bone formation rates and resorption markers than
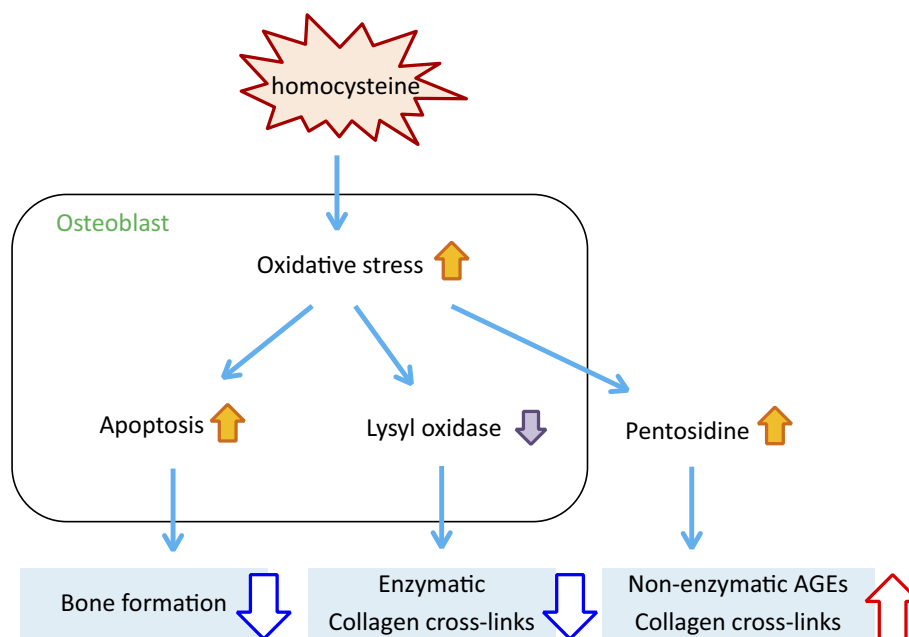
Figure 3. Effects of homocysteine on osteoblasts. Homocysteine increases intracellular oxidative stress in osteoblasts and induces apoptosis, resulting in a decreased rate of bone formation. Homocysteine suppresses the lysyl oxidase expression, which is the most important enzyme for collagen cross-links, and increases the extracellular pentosidine accumulation, resulting in the deterioration of the bone quality.

obese insulin-sensitive and lean subjects. However, the BMD in obese and insulin-resistant subjects was greater than that in obese, insulin-sensitive, and lean subjects. Taken together, insulin insufficiency/deficiency, such as T1DM and long-standing T2DM, induces bone loss and bone quality deterioration due to decreased bone formation and bone turnover, resulting in an extremely high risk of fractures. In contrast, T2DM with insulin resistance may result in bone quality deterioration without bone mass reduction due to deficiency in insulin activity in the bone.

4. Insulin-like growth factor-I

Insulin-like growth factor (IGF)-I is known to have an anabolic effect on bone. IGF-I may be involved in DM-induced bone fragility because the serum IGF-I level is known to be decreased in patients with poorly controlled diabetes (79, 80), and endogenous insulin secreted from pancreatic β cells is important for promoting serum IGF-I secretion (81, 82).

IGF-I is expressed in osteoblasts and promotes osteoblastic differentiation and bone remodeling via autocrine and paracrine pathways in the microenvironment. Circulating IGF-I is mainly produced in the liver by growth hormones and diet and acts in an endocrine manner on bone. Previous studies have shown that mice with osteoblast-specific knock-out of the IGF-I receptor had a significant reduction in the bone mass and deficient mineralization (83), and that liver-specific IGF-I gene-null mice had a marked reduction in bone volume, periosteal circumference, and medial-lateral width (84).

In addition, several laboratory studies have shown that the stimulatory actions of IGF-I on osteoblasts are inhibited by high glucose and AGEs (85, 86), and that high glucose significantly impairs the proliferative and functional responses of osteoblastic cells to IGF-I (85). AGEs also significantly decreased the IGF-I secretion in osteoblasts (86). Thus, hyperglycemia and AGEs may induce resistance of osteoblasts to IGF-I activity locally. Therefore, IGF-I signaling is important for maintaining the bone mass and strength in patients with diabetes, and decreased IGF-I levels may be involved in DM-induced bone fragility.

Indeed, several studies have shown that lower serum IGF-I levels are associated with a risk of fracture in patients with diabetes. We have previously shown that the serum IGF-I level was positively associated with serum osteocalcin levels and inversely associated with the prevalence and severity of vertebral fractures in postmenopausal women with T2 DM (87, 88). Furthermore, in a retrospective cohort study, a decreased serum IGF-I level was associated with an increased risk of non-vertebral fractures in postmenopausal women with T2DM (89). Ardawi et al. also reported a cross-sectional study showing that the serum IGF-I level in postmenopausal women with T2DM was significantly lower than that in age-matched subjects without T2DM and that it decreased according to the number of vertebral fractures in subjects with T2DM (38). Of note, the association between serum IGF-I and the risk of fracture was independent of BMD values (38, 87-89). Thus, serum IGF-I may be important for maintaining the bone quality in patients with T2DM.

Effects of Treatments for DM on Fracture Risk

Several cohort studies have shown that poor glycemic control is associated with the risk of fracture. In a prospective population-based cohort Rotterdam study, inadequately controlled diabetes (HbA1c ≥7.5%) was associated with a 1.47- to 1.62-fold higher fracture risk than adequately controlled diabetes (HbA1c <7.5%) and no DM, whereas patients with adequately controlled diabetes had a risk similar to those without DM (90). A retrospective cohort study using the national Diabetes Case Management Program in Taiwan showed that the risk of hip fracture was linearly associated with the baseline HbA1c levels and significantly increased among patients with HbA1c levels of 9-10% and > 10% up to 1.24- and 1.32-fold, respectively, compared with patients with HbA1c levels of 6-7% (91). These large-scale studies indicate that long-term exposure to hyperglycemia increases the fracture risk in patients with diabetes. Because intensive glycemic control dramatically affects bone turnover markers (27, 28, 92), treatment for DM has been suggested to reduce the risk of fracture. In the Action to Control Cardiovascular Risk in Diabetes randomized trial (intensive or standard glycemia strategies), the incidence of fracture and falls was evaluated (93). Intensive glycemia with a median HbA1c of 6.4% did not increase or decrease the fracture or fall risk compared with standard glycemia with a median HbA1 of 7.5%. Furthermore, an open-label, randomized controlled trial J-DOIT3 showed that an intensified multifactorial intervention for glucose, dyslipidemia, hypertension, and obesity did not affect the incidence of fracture in patients with T2DM compared with conventional therapy (94). Therefore, no evidence has yet supported the notion that treatments for DM can reduce the risk of fracture.

In contrast, falls and trauma subsequent to hypoglycemia may increase the incidence of fractures. In fact, several studies have shown an increased risk of fractures associated with hypoglycemia or tight glycemic control. An epidemiological study showed that a prior episode of hypoglycemia was independently associated with an increased risk of all fractures and hip fracture up to 1.69- and 1.55-fold, respectively (95). Recently, a nationwide population-based cohort study examining 2,588 patients with T2DM who had developed severe hypoglycemia and 5,173 subjects in a comparison cohort reported that the incidence of hip fracture in patients with severe hypoglycemia was significantly 1.71-fold higher than that in those without it, and that approximately half of the individuals developed fractures within 2 years from the first occurrence of severe hypoglycemia (96). In addition, a medication analysis indicated that patients taking sulfonylurea, insulin, and insulin secretagogues combined with insulin had a higher risk of hip fracture.

In the Health, Aging and Body Composition study, compared with HbA1c >8%, HbA1c <6% was associated with an increased risk of falls in elderly patients with diabetes (97). Of note, this association was found only in insulin

users, suggesting that hypoglycemic episodes may be involved in the relationship between tight glycemic control and fall risk. Furthermore, a case-control study examining the association between HbA1c, hypoglycemic medications, and hip fracture showed that patients with tighter glycemic control (HbA1c <6% and 6.1-7.0%) were more likely to have increased incidence of hip fracture (up to 3.01- and 2.34-fold) than those with HbA1c >8% (98). A prospective cohort study revealed that a baseline HbA1c level between 6.5% and 6.9% was the optimal glycemic control range with respect to fracture (99). The risk of fracture increased when the HbA1c level was either below or above this range, suggesting that both hypo- and hyperglycemia increase the fracture risk. We should therefore pay careful attention to patients with hypoglycemia in order to prevent fracture during treatments with insulin secretagogues and insulin treatment, particularly among elderly patients.

Previous studies have reported that several anti-diabetic drugs affect the bone metabolism and fracture risk (100-102). As described above, insulin has an anabolic action on bone and stimulates bone remodeling; however, patients with T2DM undergoing insulin therapy have an increased risk of fracture in clinical settings (103-105). Insulin therapy is commonly used to treat advanced T2DM, and hypoglycemia and fall risk are increased in patients being treated with insulin. These factors might be strongly associated with the risk of fractures in such patients rather than the anabolic effect of insulin on bone.

In contrast, accumulating evidence from many preclinical and clinical studies has confirmed that thiazolidinediones (TZDs), pioglitazone, and rosiglitazone increase the risk of fractures in postmenopausal women with T2DM, but not men (105-107). TZDs are peroxisome proliferator-activated receptor-γ (PPARγ) agonists and widely used to treat T2DM with insulin resistance. PPARγ is expressed in bone marrow cells and acts as a molecular switch that regulates the fate of pluripotent mesenchymal stem cells. TZDs have been shown to stimulate the differentiation into adipocytes in preference over osteoblasts and to induce bone loss characterized by a deficient osteoblast function (108, 109) (Fig. 4). Previous studies have shown that TZDs induce apoptosis and increase the sclerostin expression in osteocytes (110, 111). Mieczkowska et al. reported that TZD-induced osteocyte apoptosis was mediated through the activation of the ERK1/2 and p38 signaling pathways independently of PPARγ activation, whereas sclerostin up-regulation was mediated through PPARγ activation (111). In addition, several studies have shown that TZD-induced PPARγ activation directly enhances osteoclastogenesis through RANKL-RANK signaling and the c-fos expression (112, 113). Therefore, alternative medications should be considered for postmenopausal patients who are at an increased risk of fracture.

In contrast, a few epidemiological studies have shown that patients taking metformin might have a reduced fracture risk (103, 104). Several preclinical studies have shown that metformin enhances the differentiation of stromal cells into
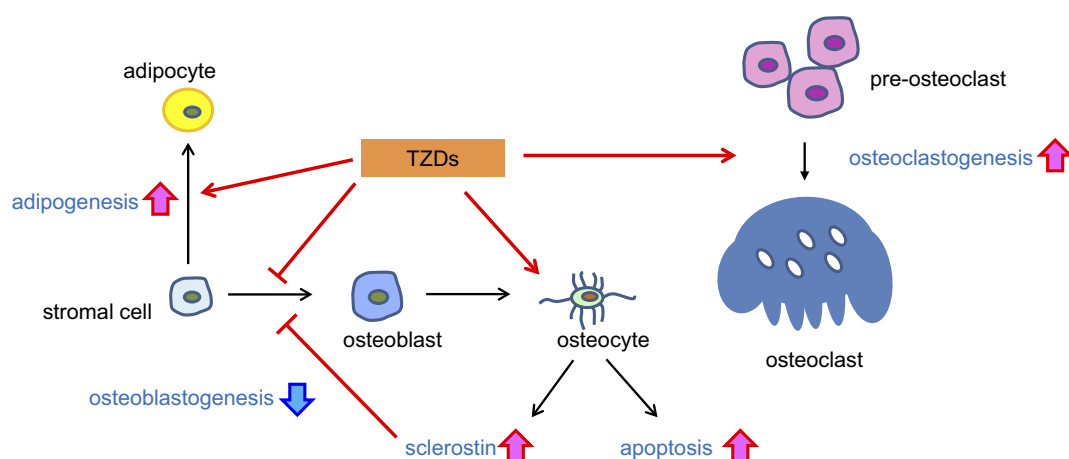
Figure 4. **Effects of thiazolidinediones (TZDs) on bone metabolism. Based on previous *in vitro* and *in vivo* studies, the effects of TZDs on bone metabolism are summarized. TZDs inhibit the differentiation of stromal cells into osteoblastic lineage and promote adipogenesis. TZDs induce apoptosis of osteocytes and increase sclerostin expression, which suppresses osteoblastogenesis by antagonizing Wnt signaling. TZDs also induce osteoclast differentiation. Taken together, these findings indicate that TZDs decrease bone formation and increase bone resorption, resulting in a reduction in bone mass.**

osteoblasts by increasing the Runx2 expression but inhibits that into adipocytes by decreasing the PPARγ expression (114) (Fig. 5). Furthermore, metformin increases the differentiation and mineralization of osteoblasts (115, 116), as well as suppresses osteoclast activity directly (117) and indirectly by the suppressing RANKL expression and increasing the OPG expression in osteoblasts (118). Metformin has also been reported to have a protective effect against apoptosis of osteocytes (68, 119) and to cancel the deleterious effects of AGEs on osteoblasts (120). These findings seem to suggest that metformin has a beneficial effect on the fracture risk in patients with T2DM, although several studies have reported no effects on bone (121, 122); therefore, further studies are needed to clarify the potential protective role of metformin.

Incretins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are gastrointestinal hormones that regulate glucose metabolism by stimulating insulin secretion, inhibiting glucagon secretion, and reducing gastric emptying and food intake. GLP-1 and GIP are rapidly inactivated by dipeptidyl peptidase-4 (DPP-4), and GLP-1 receptor agonists and DPP-4 inhibitors are now available for the treatment of T2DM in clinical settings. Although several preclinical studies have shown that these incretins might have beneficial effects on the bone mass and strength (123-125), recent clinical studies have indicated no significant clinical effect of GLP-1 receptor agonists and DPP-4 inhibitors on skeletal tissues (126-129).

In contrast, recent epidemiological studies have shown that the use of DPP-4 inhibitors in combination with metformin was associated with a decrease in the risk of fractures in patients with T2DM (130, 131). Dombrowski et al. reported that DPP-4 inhibitor use in patients with an initial prescription of metformin significantly reduced the risk of developing fractures compared to metformin monotherapy (130). Because incretin-based therapy is relatively new, clinical data on its safety or potential benefits to bone are just emerging. Furthermore, in most studies, fractures have been reported as adverse events and not as primary or secondary endpoints. Thus, further studies are necessary to determine the effects of GLP-1 receptor agonists and DPP-4 inhibitors on fracture risk.

Sodium glucose co-transporters 2 (SGLT2) inhibitors are currently the newest class of anti-diabetics. SGLT2 allows the reabsorption of glucose in the proximal tubule of the kidneys and is responsible for 90% of renal glucose reabsorption. SGLT2 inhibitors act independently of insulin action and promote a negative energy balance by increasing glycosuria. With regard to the mechanism of SGLT2 inhibitors, hypercalciuria with glycosuria and subsequent negative balance of calcium metabolism, which may result in decreased bone volume, may occur with long-term exposure to SGLT2 inhibitors. A negative balance of energy metabolism and loss of body weight may also increase the fracture risk. Indeed, a few experimental studies have shown that SGLT2 inhibitors are associated with adverse effects on bone (132). A recent large-scale randomized clinical trial using canagliflozin demonstrated that canagliflozin treatment in patients with T2DM with a high risk for cardiovascular diseases significantly increased the risk of all fractures but not low-trauma fracture (133). However, recent meta-analyses of randomized clinical trials have shown no significant difference in the fracture risk between those taking SGLT2 inhibitors and controls (134, 135). The available data were limited by the short duration of treatment and the short follow-up period, and only a low incidence of fracture was reported in previous studies. Therefore, further safety monitoring and real-world data with detailed information on skeletal health
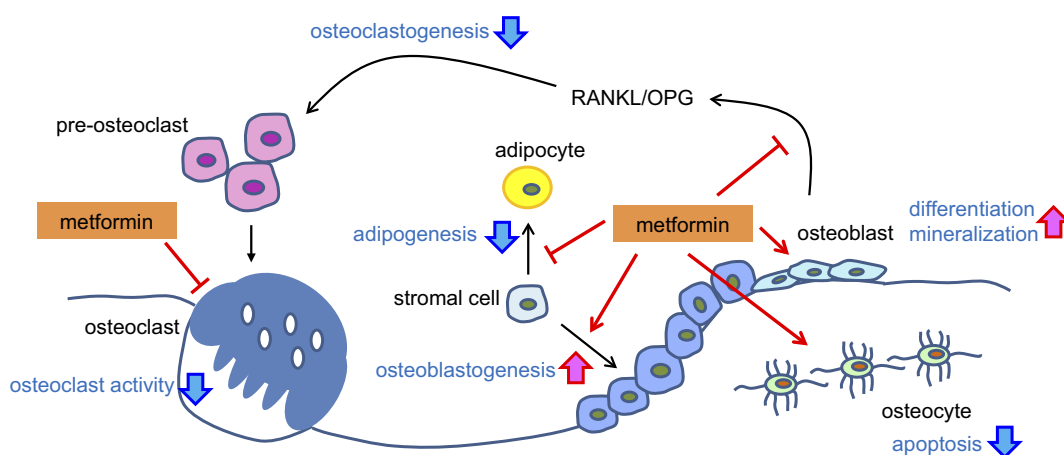
Figure 5.   Effects of metformin on bone metabolism. Based on previous *in vitro* and *in vivo* studies, the effects of metformin on bone metabolism are summarized. Metformin promotes the differentiation of stromal cells into osteoblastic lineage and inhibits adipogenesis. Metformin enhances the differentiation and mineralization of osteoblasts. Metformin protects against apoptosis of osteocytes. Metformin suppresses osteoclastogenesis by decreasing the RANKL expression and increasing the OPG expression in osteoblasts. Metformin also directly inhibits the osteoclast activity. Taken together, these findings indicate that metformin increases bone formation and decreases bone resorption, resulting in a gain in bone mass. RANKL: receptor activator of nuclear factor κB ligand, OPG: osteoprotegerin

are needed.

Fall Risk in Patients with Diabetes

Falling is a major health concern among elderly people because it increases the incidence of fractures and causes deterioration in mobility. Many clinical studies have shown that DM is associated with an increased risk of falling (136, 137); therefore, in addition to bone fragility, fall risk also causes an increased risk of fracture. Several studies have shown that the risk of falls is increased by diabetic microangiopathies, such as neuropathy and retinopathy, macrovascular diseases, as well as sarcopenia, which is a disorder characterized by the degenerative loss of skeletal muscle mass, quality, and strength and occurs more frequently in patients with diabetes than in those without diabetes (138, 139). Several common factors with DM-induced bone fragility, such as AGE and decreased insulin and IGF-I action, might be involved in the mechanism of DM-associated sarcopenia (140-142). Furthermore, osteoporotic fractures are known to be related to a decrease in physical performance and an increased risk of falls. Hip fracture is known to cause imbalance and decreased physical performance. Furthermore, the prevalence of vertebral fractures causes a change in body posture due to increased kyphosis of the thoracic spine, which is associated with decreased muscle strength in the back and lower extremities (143). Indeed, we previously reported that not only the presence of diabetic neuropathy and peripheral artery disease, but also a history of osteoporotic fractures are independently associated with fall risk in patients with T2DM (144). These findings suggest that falls and osteoporotic fractures are closely associated. Therefore, the management of patients with an increased falling risk is important for preventing osteoporotic fracture, particularly in patients with DM.

Conclusion

Emerging evidence from epidemiological, clinical, and experimental studies has shown that DM-induced bone fragility is mainly caused by the deterioration of the bone quality. Although the mechanism is not fully understood, the accumulation of AGE collagen cross-links in the bone, decreased bone formation and remodeling rates due to dysfunction of osteoblasts and osteocytes, and changes in the bone microstructure are suspected to be involved. Osteoblast and osteocyte dysfunction may be caused by complex diabetic conditions, such as hyperglycemia, high AGE and Hcy levels in the circulation, and decreased insulin and IGF-I activities. We should therefore consider DM-induced bone fragility as an important diabetic complication. Furthermore, some antidiabetic drugs may affect the bone metabolism and fracture risk in patients with DM. However, evidence suggesting which types of blood glucose management methods are most effective at improving DM-induced bone fragility is not sufficient at present. In addition, falling increases the risk of fracture, and vice versa. Therefore, developing a strategy for the treatment of bone fragility through further studies and discussions is important.

The authors state that they have no Conflict of Interest (COI).

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