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Review

Insights into bone fragility in diabetes: the crucial role of bone quality on skeletal strength

Masahiro Yamamoto

Internal Medicine 1, Shimane University Faculty of Medicine, Izumo 693-8501, Japan

Abstract. Meta-analyses have revealed that the relative risk of hip fractures in patients with type 1 and type 2 diabetes mellitus is higher than that in non-diabetic subjects. The risk of fracture in patients with diabetes mellitus increases along with a decrease in bone mineral density (BMD) similarly to those in non-diabetic patients. However, the observed risk of fracture is higher than expected one by BMD in both type 1 and type 2 diabetic patients, indicating that precise estimation of bone fragility by BMD values in patients with diabetes is difficult. Bone strength consists of BMD and bone quality, for this reason, poor bone quality is a most suitable and explicable cause for elevated fracture risk in this population. This bone fragility observed in patients with diabetes mellitus is caused by unique pathogenesis in diabetes, suggesting that osteoporosis in diabetic patients may be one of the diabetic complications and that specific diagnostic criteria for this osteoporosis is required. Bone quality indicators closely related to bone fragility are required to be identified to establish a diagnostic method for osteoporosis in patients with diabetes mellitus.

Key words: Bone quality, Low bone turnover, Vertebral fracture, Advanced glycation end-products (AGEs), Pentosidine

WITH THE DEVELOPMENT of insulin therapy for diabetic patients after the discovery of insulin in 1921, microvascular diseases, such as diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy, were identified as the complications of diabetes mellitus. In the same period, in 1948, Albright reported that diabetic patients who had been exposed to poor blood glucose over a long period developed osteoporosis for the first time. However, the underlying mechanisms of these associations remained unknown.

Bone mineral density in diabetic patients

Measurement of bone mineral density (BMD) is an established method for assessment of bone strength. The association between decreased BMD measured by dual-energy X-ray absorptiometry (DXA) and fracture rate was found in postmenopausal osteoporosis. In 1991, osteoporosis was defined as "a disease that is characterized by low bone mass, microarchitectural deterioration of bone tissue leading to enhanced bone fragility, and consequent increase in fracture risk" [1], and the diagnosis criterion of osteoporosis primarily based on bone density was established. In contrast, there was less information on BMD value of diabetic patients. Diabetes mellitus is classified into two major types: type 1 diabetes mellitus (T1DM), which is caused by a loss of ability to secrete insulin that possess anabolic action of bone, and type 2 diabetes mellitus (T2DM), which develops in the presence of underlying insulin resistance. In T1DM, BMD measured in the femoral neck or the lumbar vertebrae has been reported to be significantly lower than the respective value in age and body mass index-matched non-diabetic subjects [2, 3]; these findings were consistently confirmed in other reports [4, 5]. A meta-analysis published in 2007 showed that BMD Z-scores (the age-adjusted BMD) of hip and spine in T1DM patients was lower than those in non-diabetic participants [6]. In contrast, the BMD values at these sites in T2DM patients were inconsistent; early reports with small number of patients showed that BMD values were lower than, equivalent to, or higher than those in the control groups [7-10]. However, successive reports from large-scale studies indicated that BMD values in these subjects were significantly higher than in non-diabetic populations [11, 12]. The meta-analysis including these studies revealed that the BMD Z-score in T2DM patients was higher than those in non-diabetic population,

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Correspondence to: Masahiro Yamamoto, Internal Medicine 1, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan. E-mail: masa-ya@med.shimane-u.ac.jp

unlike in the case of that in T1DM patients [6].

The risk of fracture in diabetic patients

The relationship between the presence of diabetes and risk of fracture has been investigated by the clinical type of diabetes. In the patients with T1DM, the risk of hip fractures after adjustment for confounding factors has been reported to be significantly higher in female patients compared with that in non-diabetic subjects [13, 14]. Two meta-analyses confirmed the consistent relationship between the presence of diabetes and risk of fracture [6, 15]. In contrast, the findings obtained from the patients with T2DM confused us for a long time: some reports indicated that the risk of hip fractures is increased in T2DM. However, others showed the opposite results. Two meta-analyses concluded that the risk of hip fracture in T2DM is significantly higher than that of non-diabetic subjects, although their BMD was higher compared to the control group [6, 15]. Taken together, these findings suggest that diabetes mellitus is an underlying disease for secondary osteoporosis because the risk of fracture is increased in diabetic patients irrespective of their diabetic clinical type.

Characteristics of the risk of fracture in diabetes mellitus

Vestergaard *et al*. found out some interesting results in their report [6]. The predicted odds ratios for the relative risk of fracture in T1DM and T2DM patients, when estimated by the Z-score, were 1.42 and 0.77, respectively. However, the observed values were 6.94 and 1.38, respectively, indicating that the risk of hip fractures in the patients with diabetes was higher compared to the risk predicted by BMD, irrespective of the diabetic type. On the other hand, Schwartz *et al*. observed that the rate of hip fractures over a period of 10 years in T2DM patients aged 75 years was higher at any BMD of femoral neck than that in non-diabetic population of the same age [16]. These finding suggested that it is difficult to assess bone fragility in diabetic patients by BMD that is conventional and golden standard method for diagnosis of osteoporosis.

Occurrence of hip fracture needs excess external force such as falls. The risk of falls is increased in the diabetic patients with diabetic retinopathy, diabetic peripheral neuropathy, orthostatic hypotension, or hypoglycemia, and in those on insulin therapy with an HbA1c of < 6% [17-21]. However, the risk of fracture in T2DM patients is higher than that in non-diabetic subjects, even when statistical adjustment for the history of falls was performed [22], suggesting that the increased risk of falls is not a major factor in the increased risk of fractures in diabetic patients. On the other hand, vertebral fractures may be a susceptible sign of the presence of bone fragility, because these fractures can be caused by mild external forces generated during the course of daily activities without any obvious injury such as falls. In T2DM patients, the relative risk of vertebral fractures is significantly higher than that in non-diabetic subjects (Fig. 1), despite BMD being markedly higher than that in non-diabetic patients, irrespective of sex [23] (Fig. 2), and significant association is not found between BMD and risk of vertebral fractures [23, 24] (Fig. 3). In addition, studies in T1DM patients have shown that, unlike the control group, there is no significant relationship between fracture severity and BMD Z-score [25]. Taken together, the observed risk of fracture is higher than the estimated one by BMD of these patients [6, 15, 16, 23], suggesting that pathophysiologic mechanisms not able to be assessed by BMD underlies in bone fragility of diabetic patients, irrespective of the clinical type of diabetes.

Osteoporosis is defined as "a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture", and bone

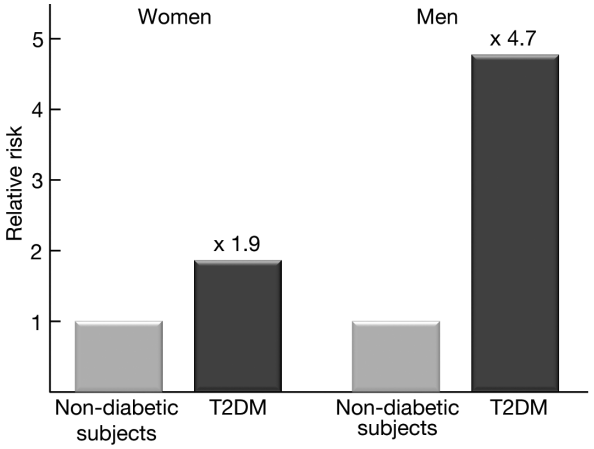

Fig. 1 Associations between the presence of T2DM and vertebral fractures
The relative risk of vertebral fractures in T2DM patients is significantly higher than that in non-diabetic subjects in both genders after adjustment for age, BMI, and $L_{2\text{-}4}$ spine BMD. (From Ref. No. 23)

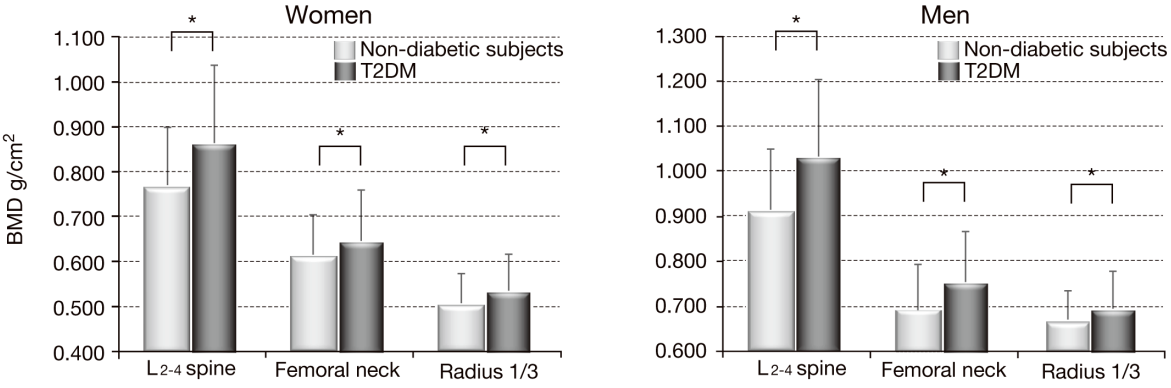

Fig. 2 Comparison of BMD between non-diabetic subjects and T2DM patients
BMD values at any site in T2DM patients are significantly higher than those of non-diabetic subjects in both genders. *, $p < 0.05$ (From Ref. No. 23)

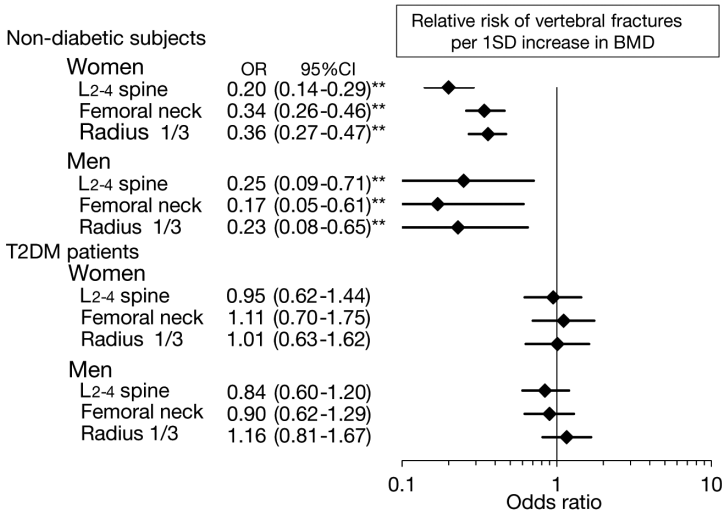

Fig. 3 Association between BMD and vertebral fractures in non-diabetic subjects and T2DM patinets
In contrast to non-diabetic subjects, the association between BMD and risk of vertebral fracture are not observed in T2DM patients. **, $p < 0.01$ (From Ref. No. 23)

strength consists of "BMD" and "bone quality" [26] (Fig. 4). Estimation of bone strength by BMD is difficult for diabetic subjects, therefore, poor bone quality is a most suitable and explicable cause for elevated fracture risk in these population, and may be a diabetes-specific mechanism for bone fragility.

Mechanism of decreased bone quality in diabetic patients

Bone quality is divided into material properties and geometrical properties (Fig. 4): the former reflects the physical characteristics of bone, and the latter indicates the morphological characteristics of bone. Material properties include the "bone matrix" composed mainly of collagen; "bone turnover," which shows the metabolic rate at which old bone tissue is absorbed and replaced by new bone tissue; "mineralization" of the bone tissue; and "microfractures", which are cracks confirmed using electron microscopy. The geometrical properties include "bone geometry" of cortical bone on a macroscopic level and "microarchitecture" of trabecular bone on a microscopic level [26]. Specific factors which are clinically associated with the risk of fracture independent of BMD are etiologic causes of deterioration of bone quality, and may serve as a powerful clue for elucidating the pathology of bone fragility in diabetic patients.

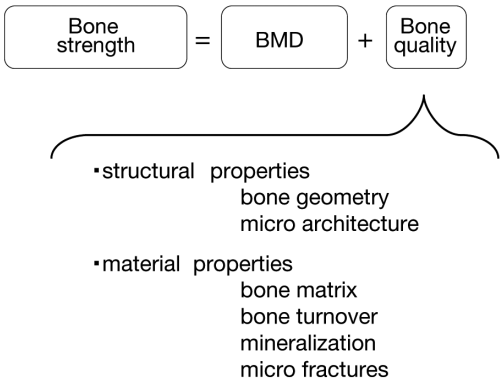

Fig. 4 Definition of bone strength
Bone strength consists of "BMD" and "bone quality", the latter includes structural properties and material properties. (From Ref. No. 26)

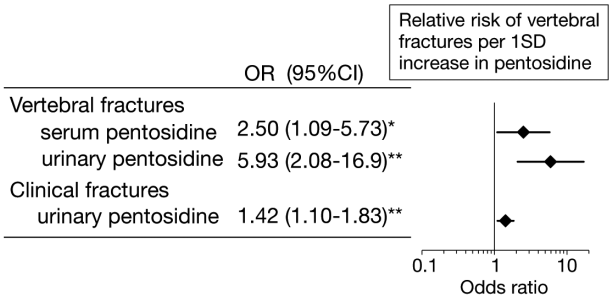

Fig. 5 The association between serum or urinary pentosidine levels and vertebral as well as clinical fractures in T2DM patients
Increased serum and urinary pentosidine concentrations were related to an increased risk of vertebral as well as clinical fractures independent of BMD after adjustment for multiple variables at least including age, BMI, HbA1c, renal function and BMD.
* $p < 0.05$, ** $p < 0.01$

Deterioration of the bone matrix and bone fragility

Type I collagen is the main constituent protein of the bone matrix; formation of cross-links between neighboring collagen molecules by enzymatic reaction changes into stabilized collagen fiber, which determine the mechanical strength of the bone tissue. Pentosidine, one of advanced glycation end-products (AGEs) which is known to be increased in diabetic patients, is composed of lysine and arginine cross-linked by a pentose. Saito *et al*. showed that pentosidine was increased in bone collagen content just before onset of diabetes in spontaneously diabetic rats and that bone strength measured by three-point bending fixture test in diabetic group was significantly decreased compared to that in control group [27, 28]. Indeed, the negative correlation between bone strength and bone pentosidine content has been confirmed in non-diabetic patients with hip fracture [29, 30]. These findings suggest that hyperglycemic condition pathologically promote the excessive glycosylation of bone collagen that is assumed to form cross-linking between collagen fibers by a non-enzymatic mechanism. This change in material properties of bone collagen may be plausible causes for poor bone quality that is deteriorated bone strength that cannot be assessed by BMD.

Bone content of pentosidine is significantly and positively correlated with its serum concentration [31]. Clinical studies showed that increased serum and urinary pentosidine concentrations were related to an increased risk of vertebral as well as clinical fractures independent of BMD in T2DM patients [32, 33] (Fig. 5). These observations indirectly suggested that advanced glycation of bone collagen in patients with diabetes also deteriorates material property of bone tissue. A microindentation method recently reported directly measures material properties of bone based on the depth of the dimple impacted by the testing probe on the tibia [34]. This method revealed that bone material strength of T2DM women was significantly lower than that of age-matched non-diabetic postmenopausal women [35], which directly revealed the presence of poor bone quality caused by deteriorated material property of bone tissue in T2DM patients.

Bone turnover and bone fragility

The collagen products as well as the factors for mineralization, which are secreted from osteoblast during its maturity, and the collagen degradation products derived from bone tissue resorption by osteoclasts are indices for bone turnover. Parathyroid hormone (PTH) and the bone formation as well as resorption markers in T2DM patients are significantly lower than those in non-diabetic subjects (Fig. 6), indicating that these patients possess suppressed bone turnover [36, 37]. The subgroup with relatively lower bone formation in addition to lower PTH levels has a higher risk of vertebral fracture independent of BMD compared to the subgroup with relatively higher these values. This finding suggests that low bone turnover accompanied with decreased bone formation causes deterioration of bone quality. Decreased ratio of osteocalcin (OC) to

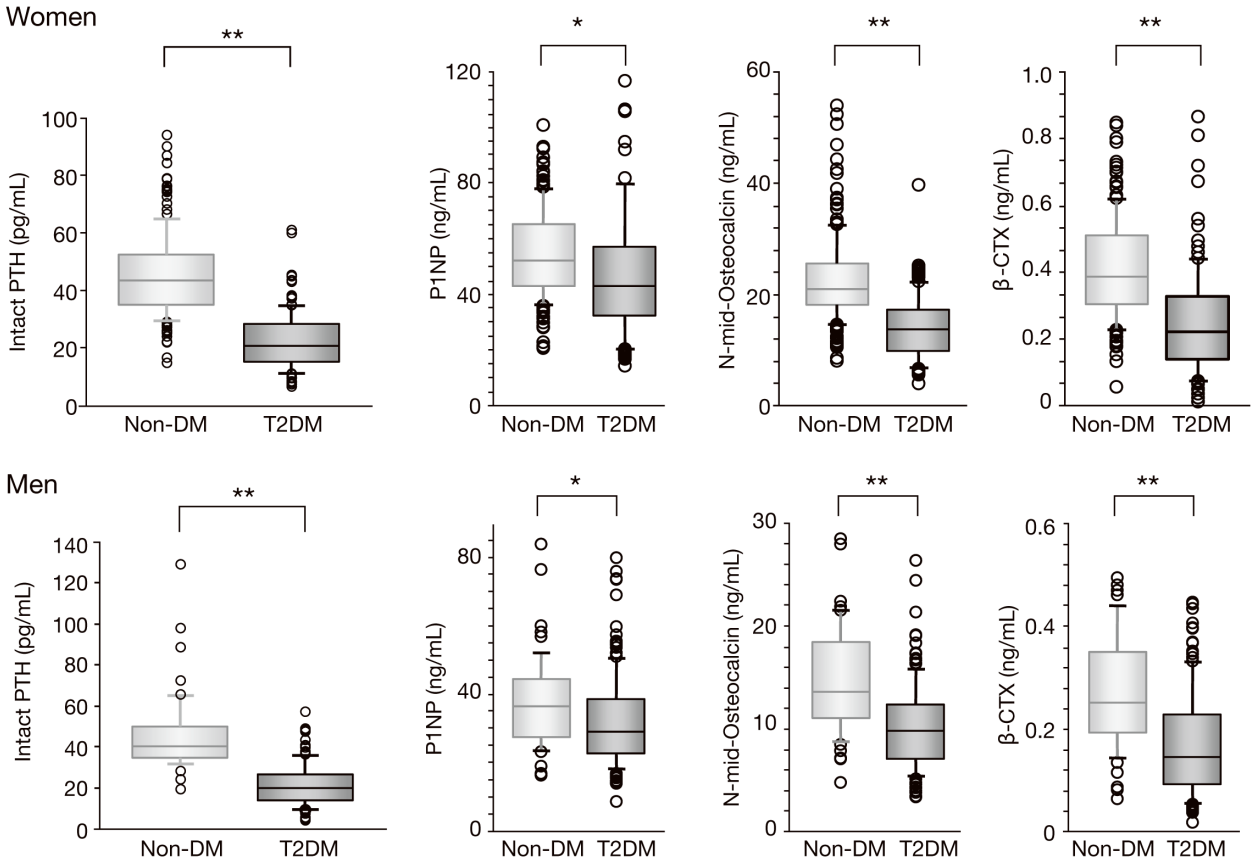

Fig. 6 Comparison of intact PTH and bone metabolic markers values between non-diabetic subjects and T2DM patients
Parathyroid hormone (PTH) and the bone formation as well as resorption markers in T2DM patients are significantly lower than those in non-diabetic subjects. Non-DM, non-diabetic subjects *, $p < 0.01$; **, $p < 0.001$ (From Ref. No. 36)

bone alkaline phosphatase (BAP), which is secreted proteins: the former from mature osteoblasts or osteocytes and the latter from pre osteoblasts, are associated with increased risk of vertebral fracture independent of BMD [38], suggesting that maturation disorders of osteoblast may be involved in the poor bone quality.

In addition, various factors for regulating bone turnover are reported to be involved in a risk of fracture independent of BMD. Insulin like growth factor-1 (IGF-1), which is abundantly present in both circulating blood and bone matrix, is an important local factor for promoting the proliferation and differentiation of osteoblasts [39, 40]. IGF-1 activates the canonical Wnt/β-catenin pathway by increasing concentration of intracellular β-catenin *via* promoting the degradation of glycogen synthase kinase-3 (GSK-3) after binding to the insulin receptor substrate (IRS)-1 [41]. The serum IGF-1 level in female T2DM patients is lower than that in non-diabetic subjects [42], which is related to an increased risk of vertebral fractures independent of BMD [42, 43].

Sclerostin is a protein secreted by osteocytes that binds to the osteoblast LDL receptor-related proteins 5 and 6 (LRP5/6) and suppresses the canonical Wnt/β-catenin pathway by inhibiting receptor complex formation. Elevated sclerostin level is significantly associated with an increased risk of vertebral fractures, independent of BMD and bone turnover [42, 44].

The receptor for AGEs, which is presented on specific cell surface, recognizes AGEs as ligands [45] and is involved in progression of diabetic complications such as diabetic nephropathy [46]. The studies on osteoblast derived from mice showed that hyperglycemia as well as AGEs suppress osteoblastic differentiation and mineralization accompanied with enhanced expression of RAGE [47-49] and that BMD was decreased in RAGE-deficient animals [50], suggesting that the AGEs–RAGE axis is involved in bone forma-

tion. Splicing variant of this receptor lacking a membrane-spanning portion is known as endogenous secretory RAGE (esRAGE), which act as "decoy receptor" inhibiting RAGE on the cell membrane from binding to AGEs outside the cell [51]. Irrespective of sex, the conditions of low esRAGE values and relatively low esRAGE values compared to AGEs are associated with an increased risk of vertebral fractures that are independent of BMD [52]. These findings suggest that AGEs are associated not only with glycation-induced physical changes to bone tissue but also with the pathogenesis of decreased bone quality through the biological effects mediated by RAGE.

These findings indicate that inhibitory factors for bone formation are associated with fracture risk independent of BMD. Under the low bone turnover coupled with low bone formation, hyper-glycosylated bone collagen or microfractures may accumulate in bone matrix. As a result of these metabolic disorders, bone fragility may increase due to deterioration of bone material properties.

Structural properties of bone and bone fragility

Bone strength of cylindrical bones such as the extremities and femoral neck rises as the external diameter and cortical bone thickness increase. The distal one-third of the radius in men with T2DM is narrower than that in non-diabetic patients [53]. In addition, diabetic patients with higher or equal HbA1c values of 7.5% have narrower external diameters of the femoral neck and a higher hazard ratio for fractures [54]. Recent progress in diagnostic imaging technology, high resolution-peripheral quantitative computed tomography (HR-pQCT), has shown that T2DM patients with fractures have significantly advanced cortical porosity at the radius as well as tibia compared to those without fracture [55, 56], and revealed that bone strength of these patients calculated by bone geometry of cortical bone is decreased. In addition, trabecular bone score (TBS), which reflects finesses of cancellous bone structure, is significantly lower in T2DM patients with major fractures than that without fracture [57], indicating that exacerbated microarchitecture also affects bone strength. Because diminished bone strength caused by bone morphology is not reflected by BMD, deterioration of structural bone quality is also considered as one of crucial pathogenesis of increased bone fragility in diabetic patients.

Diabetic therapy and bone fragility

Achieving favorable control of blood glucose may be effective in preventing fractures through keeping appropriate bone turnover, because improvement of blood glucose recovers decreased markers levels of bone formation [58, 59]. However, some antidiabetic agents have been reported to influence bone turnover negatively. Large-scale surveys indicated that insulin secretagogue and metformin were not associated with the risk of fracture, rather reduce it [60-63]. Several studies showed that patients with insulin therapy have a higher risk of fractures than those with other antidiabetic therapy [19, 60-65], which is considered as an adverse effect of insulin deficiency or poor blood glucose control. Glucose-dependent insulinotropic polypeptide (GIP) [66, 67] and Glucagon-like peptide-1 (GLP-1) [68, 69], which are called incretins, have been reported to increase bone mass in genetically modified animals. However, unlike result from first meta-analysis among short-term administration of various dipeptidyl peptidase-4 (DPP-4) inhibitors [70], a recent clinical study of long-term outcomes after treatment with certain DPP-4 inhibitors have been reported to show an increased risk of fractures [71]. In addition, results of GLP-1 treatment on increase in BMD in animal studies are inconsistent with those in clinical studies [72]. To date, established conclusion that incretins treatment decreases a risk of fractures in diabetic patients has not been obtained. Treatment with dapagliflozin, one of the sodium-glucose co-transporter 2 (SGLT2) inhibitors, did not significantly decrease BMD compared to that with metformin as control drug during two years administration [73]. In contrast, thiazolidine is known to suppress differentiation of undifferentiated mesenchymal cells into osteoblasts *via* activation of peroxisome proliferator-activated receptor gamma (PPARγ), and it results in decreasing bone formation. Meta-analyses have showed that the patients treated with thiazolidine have a significantly decreased BMD at lumbar vertebrae or femoral neck, compared with those treated with other hypoglycemic agents, irrespective of sex[74, 75], and that their fracture risk of hip, extremities, and all of osteoporotic fracture is significantly higher than those treated with non-thiazolidine agents [74, 76, 77].

Therapeutic effect of osteoporosis drugs for diabetic patients

None of the clinical studies has clarified whether osteoporosis drugs can prevent fractures in diabetic patients. When considering the pathological state of osteoporosis in diabetic patients, bone fragility in patients with diabetes may be rescued by improvement of bone formation or material properties. In the subanalysis of the MORE study, which demonstrated the preventive effect of raloxifene on vertebral fracture in postmenopausal osteoporotic women, risk of vertebral fracture in the subgroup with diabetes at baseline is lower than that with non-diabetic subgroup [78]. When a non-diabetic animal model which experimentally induced pentosidine was treated with raloxifene, bone strength recovers presumably through decreasing bone content of pentosidine [79], therefore, raloxifene administration to diabetic patients is expected to improve the material properties of the bone matrix and prevent fractures. On the other hand, the risk of fracture in diabetic patients also increases along with decrease in BMD [16], therefore, agents which is capable of increasing BMD may be useful in preventing fractures. Teriparatide, the only current agent promoting bone formation, decreased bone pentosidine content in addition to increasing BMD in non-diabetic animal model [80]. Teriparatide may be useful as a treatment for osteoporosis in diabetic patients, because of its pleiotropic effects, including recovery of impaired bone turnover in diabetic patients and improvement of bone matrix quality. On the other hand, bisphosphonates, which suppress bone absorption, increase BMD in T2DM patients whose bone turnover decreased, similarly to non-diabetic subjects [81], suggesting that these drugs may particularly posess advantage for preventing fracture in the diabetic patients with decreased BMD.

Conclusion

Statistical confirmation of increased risk of fracture in patients with diabetes reminds us that diabetes is one of crucial underlying illness for secondary osteoporosis. This increased risk of fracture may be affected by bone extrinsic factor, such as the increased risk of falls associated with diabetic complications or treatment. However, decreased bone quality may be a major cause of bone fragility in diabetic patients, because of the increased risk of atraumatic fractures such as vertebral fractures, and the presence of more excessively increased risk of fracture than expected by BMD. The bone fragility observed in diabetic patients is caused by unique pathogenesis in diabetes, suggesting that osteoporosis in diabetic patients may be one of the diabetic complications and that specific diagnostic criteria for this osteoporosis is required. Further researches are needed to develop easy tools for assessment of bone strength of diabetic patients such as bone quality markers closely related to bone fragility.

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Disclosures

None of the authors have any potential conflicts of interest associated with this research.

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