Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : September 30, 2017
Accepted : October 26, 2017
Published online : December 31, 2017

Review article

The mechanism of bone fragility in diabetes mellitus

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Abstract

It has been clarified that diabetic patients have a high risk of fractures. Fragility fractures are a serious event that not only causes a reduction in patients' activities of daily living (ADL) and quality of life (QOL), but also damages their vital prognosis. Considering that diabetes places patients at an increased risk of fracture, regardless of a patient's bone mineral density, it is thought that in the case of diabetic osteoporosis, bone quality deterioration is an important pathology which requires a deeper understanding. According to the reports presented thus far, bone quality deterioration is caused by an accumulation of advanced glycation end products (AGEs) in bone tissues, decreased bone formation and bone remodeling, and bone microstructure abnormalities. AGEs reduce bone strength by forming cross-links between collagen fibers. Additionally, in osteoblasts or osteocytes, receptor for AGEs (RAGE) is expressed, blocking their functions as biologically active substances. In the case of diabetes, the blood concentration of homocysteine, which is an inducer for oxidative stress, is increasing. This suggests the possibility of homocysteine as a cause of bone quality degeneration through an increase of oxidative stress in osteoblasts or osteocytes. An additional important factor which must be noted is the reduced effects of insulin and insulin-like growth factor-I (IGF-I) with anabolic properties working on bones. Hereafter, it will be necessary to build up a strategy in order to cure diabetes-related osteoporosis based on the patient's condition.

***KEY WORDS*:** Diabetes mellitus, osteoporosis, bone quality, advanced glycation end products (AGEs), glycative stress

Introduction

As the population is aging rapidly all over the world, it is an urgent problem to assist elderly individuals in maintaining self-control in terms of activities of daily living (ADL) as well as quality of life (QOL), seen from the viewpoint of not only the medicine but also social status. Osteoporosis, which reduces bone strength, causes fractures in the elderly via only a slight external force, and afterwards causes their ADL and QOL to worsen dramatically. Additionally, it is reported that after individuals fracture their bones due to osteoporosis, their vital prognosis worsens as well [1,2].

The goal of diabetes treatment is to maintain the QOL of patients as good and healthy individuals and to prolong their life spans. Based on the evidence hitherto accumulated, it is clear that diabetic patients have a significantly higher risk of fractures compared to non-diabetic people [3-5]. Therefore, it is important to devise a solution to reduce the risk of fractures, while also grasping the pathology of osteoporosis incorporated with diabetes.

In this article, a mechanism of diabetes that causes bone fragility and puts patients at risk of fracture will be described.

Increase in fracture risks due to diabetes mellitus

Bone strength is defined as the sum total of bone mass and bone quality [6]. Formerly, osteoporosis was thought to be a disease that reduces bone mineral density (BMD) of patients. However, considering the fact that even when no loss of BMD is observed, some patients continue to suffer from fragility fractures. Bone quality deterioration is considered to be an important factor which causes osteoporosis. Several analyses have clarified that fracture risks increase in the cases of both type 1 (T1DM) and type 2 diabetic (T2DM) patients. In addition, it has also been shown that in both types of diabetes, a greater number of fracture risks were seen than expected from the BMD which was investigated.

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According to the meta-analysis reported by Vestergaard [3], while in the case of T1DM, Z scores were lower by 0.22 in lumbar vertebrae and by 0.37 in femurs, compared to those of healthy individuals of the same sex and age (*Fig. 1*). This leads to the belief that while hip fracture risks, which were predicted due to reduced BMD, were higher by 1.42, the actual fracture risks were 6.94 times higher, which shows a much higher result than expected. Furthermore, Z scores of T2DM were found to increase by 0.41 in lumbar vertebrae and by 0.27 in femurs. Though hip fracture risks were expected to reduce by 0.77 based on BMD, they, in fact raised by 1.38. In the same way, based on the examination of a correlation between BMD and vertebral fractures in Japanese subjects, though the BMD of type 2 diabetic patients was higher compared to that of healthy people, their risks of vertebral fractures were found to also be high [4]. According to the report based on the integral analysis of three large-scale prospective studies by Schwarts *et al*., the BMD in the femoral neck compared to the hip fracture risk is 0.59 SD (standard deviation) higher in the case of diabetic females and 0.38 SD higher in the case of males, than in non-diabetics [5]. Therefore, if all of the focus is given only to BMD values, we might underestimate fracture risks. Bone quality deterioration is as important a factor as increase in fracture risks apart from BMD.

There was no report showing the result that fragility fractures affected ADL and QOL, as well as vital prognosis regarding diabetics. We conducted a survey using the Barthel index and SF-36 towards type 2 diabetic patients, in order to laterally examine the correlation between vertebral fractures and ADL/QOL. As a result, it was reported that in the case of patients suffering from Grade 2 and 3 vertebral fractures based on the Genant classification [7], there was a significant influence of the fractures regarding their ADL and QOL (particularly in terms of body pain, general health, vitality, social functioning and emotional role), even after adjusting for confounding factors such as age, sex, HbA1c, and renal function [8]. In addition, based on the observational study on type 2 diabetic patients, it was clarified that by suffering from multiple vertebral fractures or Grade 3 vertebral fractures, the all-cause death of type 2 diabetic patients increased despite a correction using a variety of confounding factors (*Fig. 2*) [9]. All of these results show that it is important for diabetic patients to avoid fragility fractures.

Pathology of bone quality deterioration in diabetes mellitus

Regarding the mechanism of bone quality deterioration, an accumulation of AGE cross-links between collagen fibers, as well as abnormal bone microstructure, are considered important. The bone matrix includes abundant type 1 collagens, and by forming physiological cross-links between collagen fibers, bones are able to maintain flexibility and strength. AGEs are generated by sequential nonenzymatic chemical glycoxidation of the protein amino groups. When patients suffer from diabetes, AGE cross-links are formed non-physiologically, which lowers the flexibility of collagens and causes a decrease of bone strength (*Fig. 3*).

According to a report by Saito *et al*. which examined the rats with a natural onset of diabetes, an accumulation of AGE cross-links in bone tissues was reported to cause a reduction in bone strength [10], while an increase of pentosidine, which is one of the well-known AGEs and chemically well defined, was seen in T1DM patients during the clinical study of bone biopsy [11]. Therefore, it can be assumed that the pathology of bone quality deterioration due to diabetes is linked to a non-physiological accumulation of AGE cross-links in the bones. All these are hypotheses that reflect the pathology of diabetes-related osteoporosis where bone fragility was observed without showing a reduction in BMD. It has been reported that the abnormal bone micro-structure seen in diabetic patients correlates with the cortical bone porosity

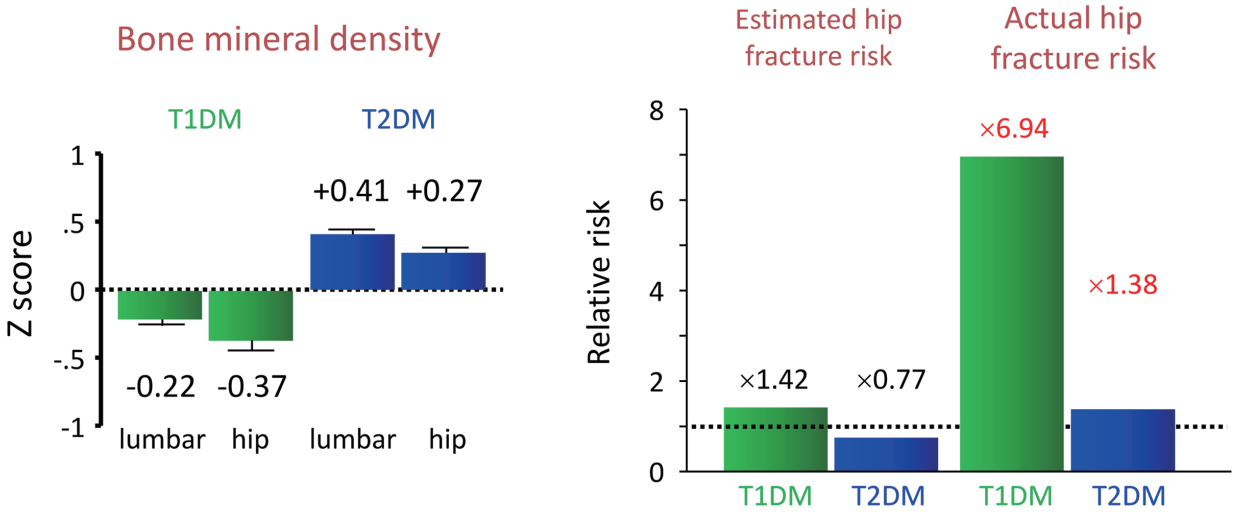

Fig. 1. **Bone density and fracture risks regarding diabetic patients.**

In the case of type 1 diabetes (T1DM), Z scores, compared to those of healthy people of the same age and the same sex, are lower by 0.22 regarding lumbars, and 0.37 regarding femurs, while in the case of type 2 diabetes (T2DM), Z scores are on the rise by 0.41 regarding lumbars, and by 0.27 regarding femurs. While it is expected that the risks of proximal femoral fractures to be 1.42 times higher in the case of T1DM, and 0.77 times higher in the case of T2DM, the actual fracture risks were each 6.94 times and 1.38 times the risk for T1DM and T2DM which were higher than expected. Author's drawing based on Reference 3.

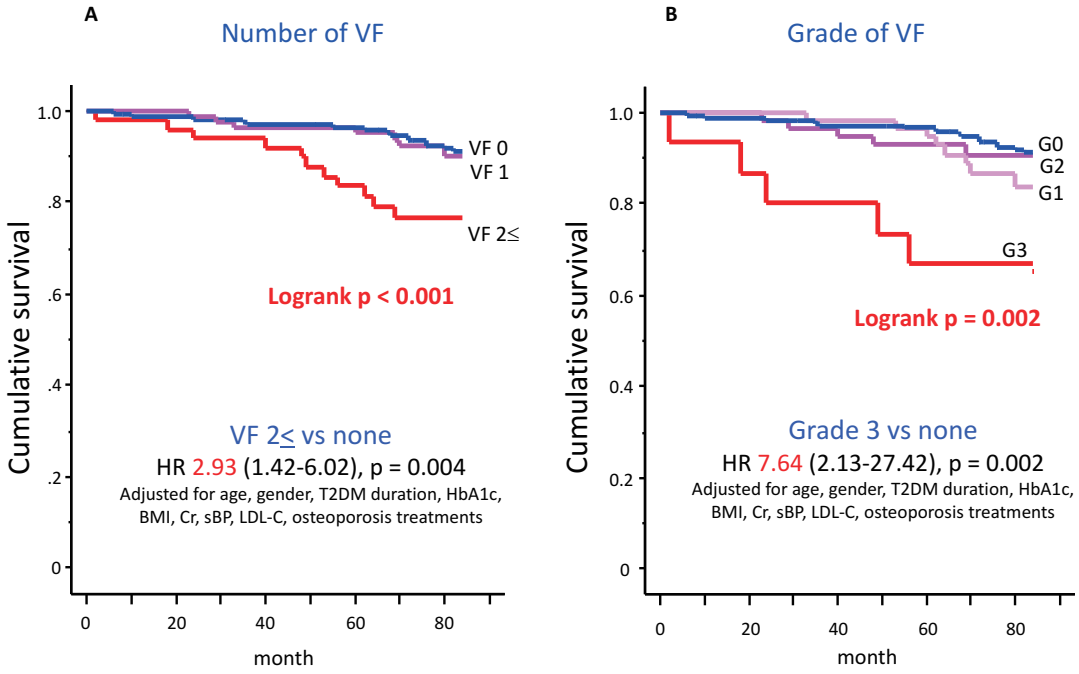

Fig. 2. **Correlation between vertebral fractures (VF) and the overall mortality rate in patients with T2DM.**

When patients have more than two VF, their cumulative survival rate was sure to lower significantly (**A**). This correlation was significant also after adjusting for age, gender, duration of T2DM, HbA1c, BMI, serum Cr, sBP, LDL-C, and treatment of osteoporosis (HR 2.93, 95%CI 1.42-6.02, p = 0.004). When VF became grade 3 (G3), the cumulative survival rate lowered significantly (**B**). This correlation was significant also, after adjusting for the confounding factors (HR 7.64, 95%CI 2.13-27.42, p = 0.002). VF, vertebral fractures; T2DM, type 2 diabetes mellitus; BMI, body mass index; Cr, creatinine; sBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HR, hazard ratio; CI, confidence interval.

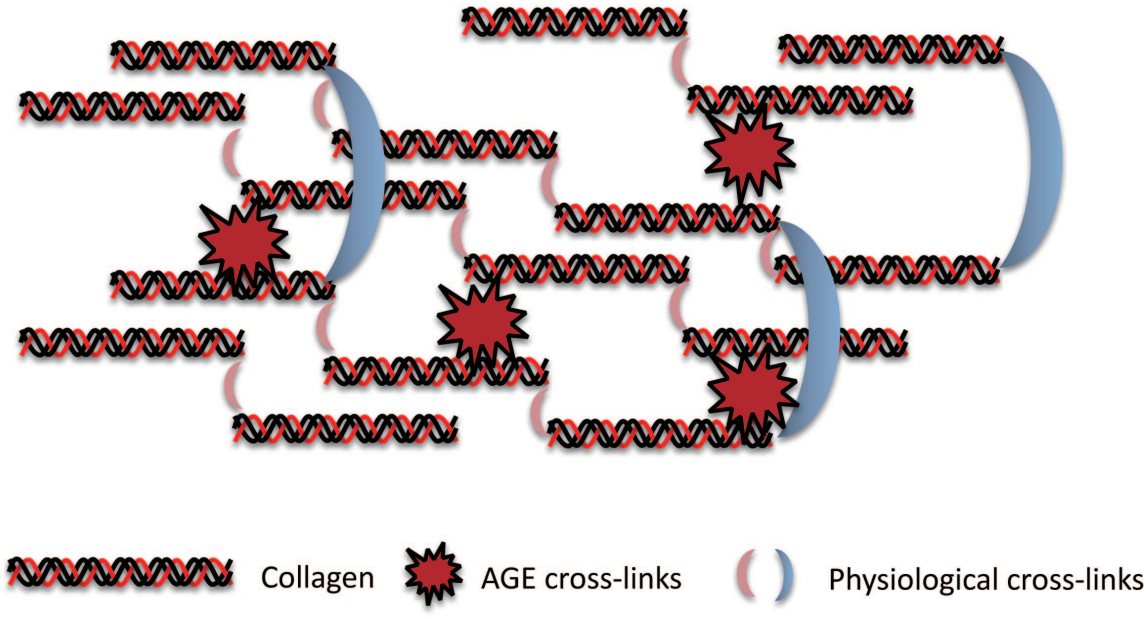

Fig. 3. **Deterioration of collagen cross-links in diabetes mellitus.**

Crosslinkings physiologically formed in collagen arrays enhance bone strength. On the other hand, non-physiologically formed AGE crosslinkings, which damage suppleness of bones, play a part in lowering bone strength. AGE, advanced glycation end product.

or the abnormal cancellous bone microstructure. According to a clinical examination using high-resolution peripheral quantitative computed tomography (HR-pQCT), cortical bone porosity is considered to be an important factor that influences bone strength regardless of BMD, which is quite remarkable [12].

It has been reported that when examining T2DM, the porosity of cortical bones was found to increase in patients suffering from fragility fractures [13, 14], which shows that the porosity of cortical bones may be correlated with the pathology of diabetes-related osteoporosis. On the other hand, it is not always the case that the porosity of cortical bones is found in all parts. In some parts, no change or even less cortical porosity was reported regarding the case of type 2 diabetic patients [14, 15], which means there should be further consideration into whether there is a correlation between the porosity of cortical bones and diabetes-related bone fragility.

Trabecular Bone Score (TBS) is an index to measure the distribution of cancellous bone microstructure. It can be used to measure fracture risks regardless of BMD. In TBS measurement, images obtained from a dual energy X-ray absorptiometry (DXA) are used for re-analysis, which means additional invasion is not necessary. In this sense, this measurement is expected to be used in the clinical application in the near future. Previously, TBS was found to be lower in type 2 diabetic patients than non-diabetes, while in the case of diabetic patients alone, lower TBS is related to fracture risks [16]. Iki *et al*. reported that in the examination on 1,683 Japanese males, lowering TBS was significantly associated with increased blood glucose levels and insulin resistance in diabetic patients [17]. Therefore, it is also possible that there is a linkage between diabetes-related osteoporosis and abnormal cancellous bone microstructures.

Though the mechanism triggering bone microstructure abnormalities in diabetes has yet to be clarified, we consider that abnormalities of osteoblast and osteocyte functions may be involved in impaired bone formation and bone remodeling, leading to bone microstructure abnormalities. Furthermore, osteocyte apoptosis may be associated with cortical porosity in diabetes-related osteoporosis.

Mechanism of osteoblast differentiation disorders in diabetes mellitus

The homeostasis of bones is maintained through a balance between bone formation by osteoblasts and bone resorption by osteoclasts. Osteoblasts not only express bone-specific alkaline phosphatase (BAP) and type 1 collagen, but also form bones by causing calcification and deposition of calcium or phosphorus. Moreover, osteoblasts produce lysyl oxidase (LOX) which is essential in the formation of physiological cross-links of collagen. In this sense, osteoblasts play an important role in maintaining bone strength and quality.

It is considered that in the case of diabetes, maturation of osteoblasts is suppressed during the initial to the late stage of differentiation. No significant difference is seen in BAP, which is a marker in the initial stage of differentiation, between diabetics and non-diabetics; however, regarding osteocalcin, which is a marker in the late stage, a significant decrease can be observed [18]. Previous studies have shown that BAP decreases and osteocalcin increases after short-term diabetes treatments [19, 20], and that the rate of osteocalcin/BAP is inversely associated with the risk of fractures [21]. These findings suggest that diabetes causes osteoblast differentiation disorders. The mechanism of osteoblast differentiation disorders is reported to show that factors such as high blood glucose, AGEs, and homocysteine play important roles.

1) AGEs

AGEs have a physiological activity: they not only form collagen cross links, but also works through receptor for AGEs (RAGE). As RAGE is also expressed in the osteoblasts, AGEs work on osteoblasts directly. As a result, AGEs cause apoptosis induction as well as inhibition of differentiation and mineralization. A mechanism that we clarified in the course of our research was: an acceleration of RAGE expression in the hyperglycemia state induces the enhancement of AGEs signaling [22]; AGEs inhibit mineralization from occurring remarkably through decreased expression of Runx2 and osterix that play important roles in osteoblast differentiation [23, 24]. Moreover, we reported that a dysfunction of endoplasmic reticulum stress and an increase in transforming growth factor-β (TGFβ) expression play pivotal roles in this mechanism (***Fig. 4***) [24, 25].

2) Homocysteine

Homocysteine is an amino-acid that plays an active role in the formation of methionine, which is an essential amino-acid, as well as cysteine. It is well known that excessive homocysteine induces oxidative stress. Regarding diabetes, it is thought that blood homocysteine concentration rises under the condition of a vitamin B insufficiency/deficiency caused by gluconeogenesis acceleration in the liver due to decreased insulin action. Li *et al*. reported a cross-sectional study on a linkage between blood homocysteine and fracture risks in 124 diabetic patients and 115 non-diabetics [26]. Diabetic groups showed a higher blood homocysteine concentration increase compared to non-diabetics. On the other hand, diabetics who had a history of fractures showed a significantly higher blood homocysteine concentration than those without such experiences. Furthermore, a logistic regression analysis adjusted for age, sex, vitamin B12, folic acid, and renal function clarified that high homocysteine levels in the blood was significantly and independently associated with the fracture risks.

By using osteoblast-like cells MC3T3-E1, we proved that homocysteine induced apoptosis by accelerating oxidative stress in osteoblasts, and that even when the concentration was not high enough to induce apoptosis, it inhibited LOX expression and enhanced accumulation of extracellular AGEs [27]. Therefore, it can be assumed that homocysteine causes bone quality deterioration under the condition of reduced osteoblast function and abnormal collagen cross-links (lowered physiological cross-links and increased AGE cross-links) through strengthening oxidative stress by affecting osteoblasts directly [28].

3) Insulin and Insulin-like growth factor-I (IGF-I)

It is thought that insulin action plays an important role in differentiation of osteoblasts and production of collagen. Regarding the examination using osteoblast-specific insulin

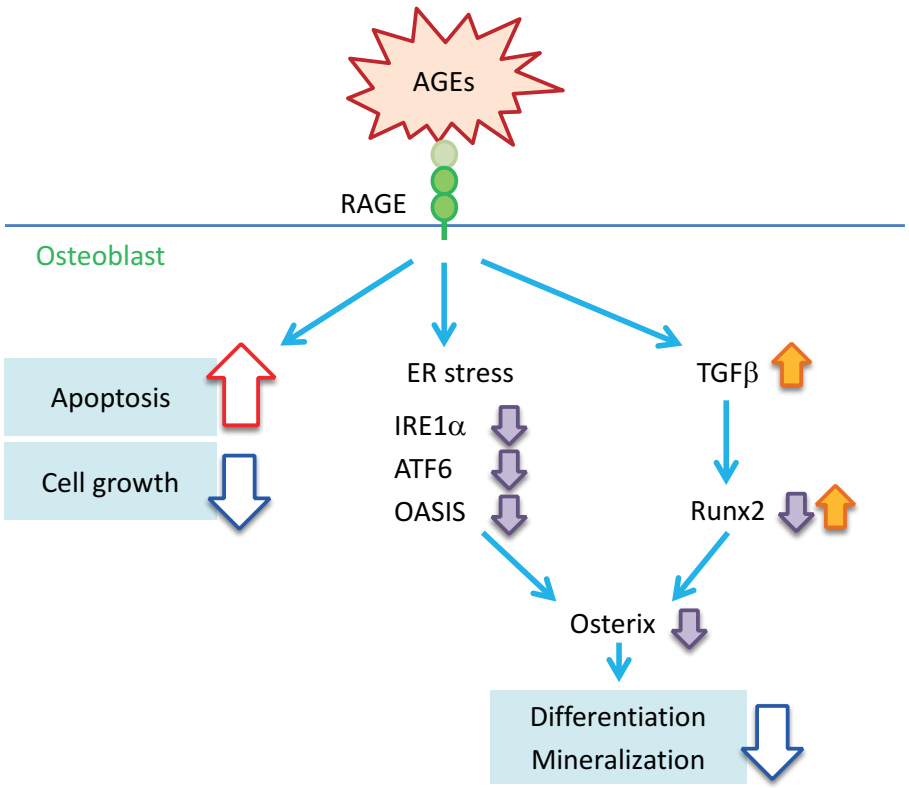

Fig. 4. **Impacts of AGEs on osteoblasts.**

While AGEs accelerate apoptosis via RAGE that exists in osteoblasts, they suppress the proliferation of cells. Additionally, AGEs suppress differentiation and mineralization of osteoblasts in their mechanism. During the process, the abnormality of endoplasmic reticulum stress proteins and increase of TGFβ expression are seen. Although AGEs suppress Runx2 expression in the initial stage of differentiation, they suppress differentiation of osteoblasts by strengthening them in the later stage of differentiation. AGEs, advanced glycation end products; RAGE, Receptor for AGEs; ER, endoplasmic reticulum; IRE1α, inositol-requiring enzyme 1α; ATF6, activating transcription factor 6; OASIS, old astrocyte specifically induced substance; TGFβ, transforming growth factor-β.

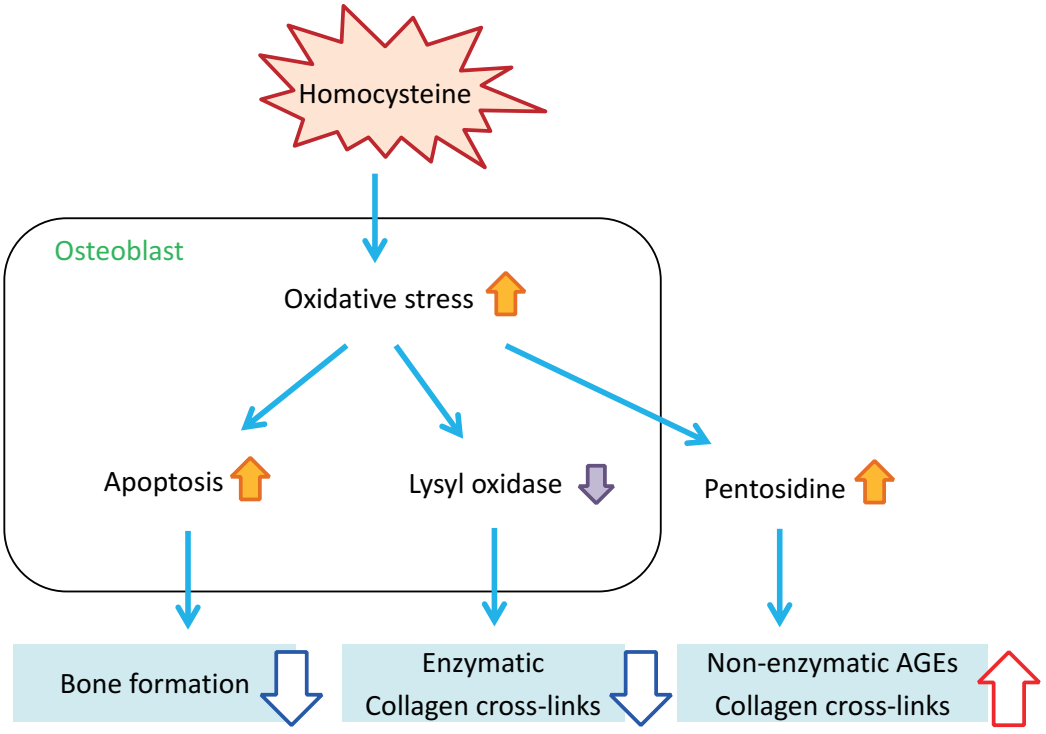

Fig. 5. **Impacts of homocysteine on osteoblasts.**

Homocysteine induces osteoblasts apoptosis, resulting in inhibition of function of osteoblasts. Furthermore, it prevents physiological collagen from forming cross-links outside cells and increases extracellular AGE cross-links. AGE, advanced glycation end product.

receptor knockout mice, it is reported that significant bone loss is induced under the condition of attenuating bone formation caused by a decrease in the number of osteoblasts [29, 30]. It has been clarified that a suppression of proliferation and an induction of apoptosis were caused by an inhibition of insulin signaling in osteoblasts and that by enhancing the production of Twist2, which is an inhibition factor of Runx2, differentiation of osteoblasts is suppressed [30]. In addition, it has also been reported that in the cases of knockout mice models of insulin receptor substrate-1 (IRS-1) or IRS-2, both of which are downstream molecules of insulin signaling, bone loss accompanying a decrease in bone formation has been observed [31]. Considering the clinical image that in the case of type 1 diabetics, a reduction in BMD with decreased bone formation is observed, it can be confirmed that insulin affects bones as an anabolic reaction. Furthermore, IGF-I has long been known as a hormone that affects bones as an anabolic reaction. IGF-I is important as a local factor, because it is also produced in osteoblasts. On the other hand, blood IGF-I that is mainly formed and secreted in the liver by the stimulus of growth hormone, affects bones as an endocrine hormone. Based on the fact that a significant decrease of mineralization and bone mass is observed in osteoblast-specific IGF-I receptor-deficient mice [32], along with a significant decrease of bone loss in the analysis of liver-specific IGF-I knockout mice [33], it is recognized that IGF-I plays an important role as an endocrine hormone. We have reported that regarding type 2 diabetic postmenopausal women, there is a significant positive correlation between blood IGF-I values and bone formation markers, and that there is a significant association between lower IGF-I values and an increase in the risk of vertebral fractures / multiple vertebral fractures [34, 35]. Additionally, it has been reported that AGEs reduce IGF-I signaling by inhibiting the expression of IGF-I and IGF-I receptor [36-38]. Based on these findings, it may be considered that lowering IGF-I activity as an endocrine hormone and inhibiting IGF-I signaling in local bones will play an important role in the pathology of bone fragility in diabetes.

Mechanism of osteocyte function abnormalities in diabetes mellitus

Osteocytes, which account for more than 90% of cells in bone, constitute the majority of all the cells in bones. Osteoblasts change into osteocytes as differentiated forms. During the process they are embedded into the bone matrix which they themselves secreted. Many osteocytes are embedded in the cortical bones. They extend their dendrites into the tunnel-like structure called canaliculi, exchanging information with adjacent osteocytes, osteoblasts, and osteoclasts.

Furthermore, osteocytes secrete humoral factors. By expressing sclerostin and Dickkopf-1, which are inhibitory factors of osteoblast differentiation, as well as the receptor activator NF-kB ligand (RANKL) and osteoprotegerin which are regulatory factors of osteoclasts, osteocytes regulate the coupling of osteoblasts and osteoclasts. Therefore, the function of osteocytes is considered important in bone metabolism control and bone remodeling. Bones become new bones every three to four months, through repeated remodeling. However, in the case of diabetes, as a bone remodeling function is decreasing, AGE cross-links and micro crackles are apt to accumulate.

Considering that RAGE is expressed in osteocytes, there is a possibility of AGEs directly affecting osteocytes. In our report on an examination using osteocyte-like cells MLO-Y4-A2, we have shown for the first time that AGEs induce apoptosis of osteocytes through RAGE, enhance sclerostin expression, and suppress RANKL expression [39]. Furthermore, we have demonstrated that apoptosis induction and enhancement of sclerostin expression by AGEs were through increased TGFβ signaling [40]. Moreover, we have reported that homocysteine directly affects osteocytes, inducing apoptosis through enhancement of oxidative stress [41]. In addition, Vijayan *et al*. reported that by administering homocysteine to mice for 30 days, apoptosis of osteocytes was induced, the number of cortical bone voids was increased, the number of sclerostin-positive osteocytes was increased, and the biomechanical property (Young's modulus) was reduced [42]. Based on these facts, it is considered possible that the effect of homocysteine on osteocytes has a relationship with an increase of fracture risks due to the hyperhomocyteinemia.

Although it is not clear how much osteocyte function abnormalities induce bone fragility in the case of diabetes, it is considered possible that osteocyte apoptosis in cortical bones might affect cortical porosity. Additionally, it is also considered that due to the bone remodeling suppression via sclerostin or RANKL, it is not possible to metabolize old bones where AGE cross-links and micro crackles are accumulated, which as a result, causes bone quality deterioration (***Fig. 6***).

Impact on osteoclasts in the case of diabetes

As described above, a mechanism for bone formation and bone remodeling decrease in diabetic patients is gradually becoming clear; however, no definite opinion about osteoclasts activity or bone resorption in the case of diabetes has been shown. Although the bone resorption marker was on the rise regarding diabetic patients based on the previous studies, a recent meta-analysis showed a significant decrease in bone resorption marker, compared to non-diabetics [43]. Some reports mention that AGEs and homocysteine suppress induction of differentiation on osteoclasts [44], and others report that they strengthen activation [45, 46]. There are various opinions. Considering that the extent of BMD reduction of diabetics is not high [3], it is difficult to think that bone resorption is increasing remarkably. It is thus assumed that bone resorption is slightly and relatively increasing, compared to decreased bone formation. Further studies are necessary to clarify this point.

Conclusions

It is becoming clear that bone fragility due to bone quality deterioration is an important pathology in the case of diabetes. Considering abnormal collagen cross-links due to AGEs, as well as functional disorders of osteoblasts and osteocytes are considered to be serious problems. It can be said that it is quite important to care for long-term blood glucose management and to decrease oxidative stress while inhibiting AGEs formation in order to prevent / improve bone fragility factors. In addition, an increase of homocysteine and oxidative stress induced by diabetes not only enhances AGEs

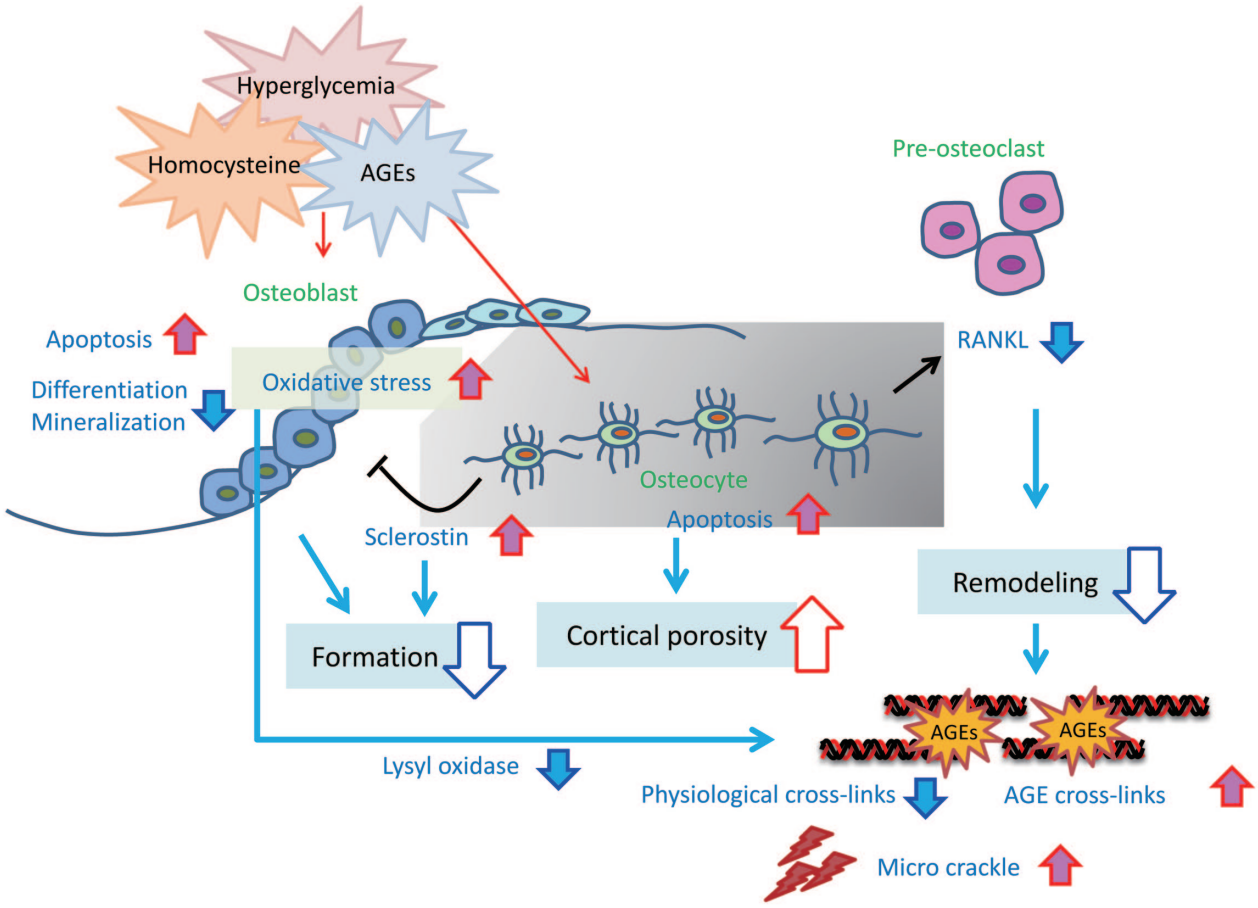

Fig. 6. **Mechanism of bone fragility caused by diabetes mellitus.**

In the case of diabetic patients, blood concentrations of AGEs and homocysteine increase. AGEs and homocysteine cause apoptosis or suppression of differentiation regarding osteoblasts, resulting in reducing bone formation rate. Furthermore, they suppress osteoblast differentiation by enhancing sclerostin expression in osteocytes. On the other hand, it causes RANKL expression to lower from osteocytes, which suppresses osteoclasts differentiation, resulting in damaging of remodeling. Owing to this, old bones which should be metabolized will not be resorbed, resulting in accumulation of AGE cross-links, and micro crackles. It can be thought that an increase of apoptosis in osteocytes induces porosity in cortical bones. AGEs, advanced glycation end products; RANKL, receptor activator of nuclear factor kappa-B ligand.

formation but also causes a reduction in bone formation and bone remodeling abnormalities. Furthermore, the endocrine environmental, abnormalities due to the reduced effects of insulin and IGF-I, is also thought to be important.

Although a mechanism of bone fragility caused by diabetes is gradually becoming clear, there are not enough considerations for which types of blood glucose management methods are effective at improving bone fragility, as well as which osteoporosis drugs are the most effective in clinical settings. All these show the status-quo where evidence is lacking. Based on the fact that osteoporosis is a disease accompanied by diabetes, which is linked to the prognosis in addition to lowering of ADL and QOL, it is an urgent task to construct a strategy for the treatment of diabetes-related osteoporosis through further discussions.

Conflict of interest

The authors have no conflicts of interest.

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