Interaction between bone and glucose metabolism

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Abstract. Accumulating evidence has shown that bone and glucose metabolism are closely associated with each other. Since the risk of osteoporotic fractures is increased in patients with diabetes mellitus (DM), osteoporosis is recently recognized as one of diabetic complications, called DM-induced bone fragility. Previous studies showed that collagen cross-links of advanced glycation end products (AGEs) and dysfunctions of osteoblast and osteocyte are involved in DM-induced bone fragility. Circulating levels of AGEs and homocysteine are increased in patients with DM, and they directly impair the functions of osteoblast and osteocyte, resulting in decreased bone formation and bone remodeling. On the other hand, bone is recently recognized as an endocrine organ. Previous studies based on in vitro and animal studies showed that osteocalcin, which is specifically expressed in osteoblasts and secreted into the circulation, may regulate glucose homeostasis. Although several clinical studies reported the relationship between osteocalcin and glucose metabolism, further large-scale and intervention studies are necessary to confirm the beneficial effects of osteocalcin on glucose metabolism in human. It has been shown that adenosine monophosphate-activated protein kinase (AMPK), an intracellular energy sensor, is involved in bone metabolism. Adiponectin and metformin stimulate osteocalcin expression and the differentiation of osteoblasts via AMPK activation. Also, AMPK activation protects against oxidative stress-induced apoptosis of osteocytes. These findings suggest that AMPK in osteoblasts and osteocytes may be a therapeutic target for DM-induced bone fragility.

Key words: Diabetes mellitus, Osteoporosis, Osteocalcin, Advanced glycation end products, Adenosine monophosphate-activated protein kinase

Introduction

The incidence of osteoporosis and type 2 diabetes mellitus (T2DM) is known to increase in prevalence with aging. However, both diseases were traditionally viewed as separate entities. Previous studies have shown that patients with type 1 diabetes mellitus (T1DM) and T2DM have an increased risk of osteoporotic fractures [1-3]. Therefore, osteoporosis is recently considered as one of diabetic complications, namely DM-induced bone fragility. Moreover, the increased risk of fracture in diabetes is independent from bone mineral density (BMD) reduction [3], suggesting that DM-induced bone fragility is mainly caused by deterioration of bone quality. Regarding the underlying mechanism of bone quality deterioration, recent studies have demonstrated that advanced glycation end products (AGEs) and homocysteine (Hcy), which circulating levels are higher in patients with DM than non-diabetes subjects, affect bone metabolism [4,5]. Further, insulin and insulin-like growth factor-I (IGF-I) action as well as adiponectin may be involved in the pathogenesis of DM-induced bone fragility [6-9]. On the other hand, bone has been recognized as an endocrine organ. Osteocalcin is specifically expressed in osteoblast lineage cells and secreted from bone into the circulation. Previous studies have shown that osteocalcin, especially undercarboxylated form of osteocalcin, is involved in whole body glucose metabolism [10]. Therefore, it is now considered that there is a close interaction between bone and glucose metabolism. Previous studies showed that activation of adenosine monophosphate-activated protein kinase (AMPK) stimulates osteoblastic differentiation and osteocalcin expression [11-13]. Also, AMPK activation protects against Hcy-induced apoptosis of osteocytes [14]. Therefore, AMPK could be a therapeutic target for DM and DM-induced bone fragility.

DM-induced bone fragility

DM is known to cause various complications such as neuropathy, retinopathy, nephropathy, and
cardiovascular diseases, resulting in the deterioration of quality of life and life expectancy. Accumulating evidence has shown that the risk of osteoporotic fractures is significantly increased in not only T1DM but also T2DM. A previous meta-analysis showed that patients with T1DM had slightly decreased BMD at lumbar spine and hip (z-score -0.22 and -0.37, respectively), and that patients with T2DM had higher BMD at lumbar spine and hip (z-score +0.41 and +0.27, respectively) [1]. According to the BMD values, the estimated fracture risks were 1.42-fold increase in T1DM and 0.77-fold decrease in T2DM. However, the risks of hip fracture compared to non-diabetes controls were 6.94-fold increase in T1DM and 1.4-fold increase in T2DM patients. Moreover, another meta-analysis also showed that hip fracture risk of T1DM and T2DM patients were increased up to 6.3-fold and 1.7-fold, respectively, compared to non-diabetes controls. Furthermore, we previously demonstrated that the presence of T2DM was an independent risk factor for prevalence vertebral fracture in Japanese men and women after adjustment for age, body mass index, and lumbar BMD [3]. These findings suggest that the increased risk of fracture is mainly caused by deterioration of bone quality but not BMD reduction, and that BMD measurement may be less useful in patients with DM compared to non-diabetics.

**Mechanism of DM-induced bone fragility**

Previous studies have shown that accumulation of AGEs in bone is involved in the BMD-independent bone fragility in diabetic patients. AGEs are generated by sequential nonenzymatic chemical glycoxidation of protein amino groups. AGEs are known to be accumulated in various tissues including bone, kidney, brain and coronary artery atherosclerotic plaques with aging. AGEs are considered to play a pivotal role in the development of diabetic complications because hyperglycemia and oxidative stress accelerate AGEs formation. Indeed, several studies showed that serum AGEs levels were significantly higher in diabetic patients than those in healthy subjects. Among AGEs, pentosidine is a well-characterized compound and is considered as a good predictor for the development of micro- and macro-vascular complications in diabetic patients [15]. Saito et al. previously showed that spontaneous diabetic rats displayed a significant increase in pentosidine collagen cross-links in bone, which was linked to impaired mechanical properties despite normal bone mass [4]. Because circulating pentosidine levels are correlated with content of pentosidine in cortical bone, serum and urine pentosidine levels could be used as a surrogate marker for its content in bone as well as bone strength. Schwartz et al. previously showed that higher urine pentosidine was significantly associated with increased clinical fracture incidence in elderly patients with T2DM in an observational cohort study [16]. Also, we reported a cross-sectional study showing that serum pentosidine levels were significantly and positively associated with prevalent vertebral fracture in postmenopausal women with T2DM [17]. In addition, a recent clinical study using bone biopsy in patients with T1DM showed that pentosidine content in trabecular was significantly and positively associated with HbA1c levels, and increased in T1DM patients with fracture [18]. Therefore, accumulation of pentosidine collagen cross-links in bone may be major cause of BMD-independent bone fragility in patients with DM.

Suppression of bone remodeling is also considered to be involved in the bone fragility in diabetic patients. Bone tissue is constantly renewed by a balance between osteoblastic bone formation and osteoclastic bone resorption. Thus, when bone remodeling process is disturbed, old bone tissue including AGEs collagen cross-links will not be renewed, leading to the deterioration of bone quality. Several clinical studies and meta-analysis have shown that bone formation markers, especially serum osteocalcin, are significantly decreased in patients with diabetes compared to non-diabetes subjects [19,20]. We previously reported that serum osteocalcin levels were increased after intensive glycemic control in T2DM, while bone specific alkaline phosphatase (BAP) was decreased [21]. In addition, the ratio of osteocalcin/BAP was significantly associated with prevalent vertebral fracture in patients with T2DM [22]. 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 diabetic patients. On the other hand, 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 canonical Wnt
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1. AGEs

As described above, AGEs are involved in DM-induced bone fragility. In addition to the accumulation of AGEs collagen cross-links in bone, it is reported that AGEs directly affect bone forming cells. AGEs have a physiological function and act through their receptors. It has been shown that receptor for AGEs (RAGE) is expressed in osteoblasts and osteocytes [25,26], and hyperglycemia increased the expression of RAGE [27]. We previously demonstrated that combination of high glucose and AGEs inhibited the mineralization of osteoblastic MC3T3-E1 cells [27], and that AGEs inhibited the osteoblastic differentiation or mineralization of mouse stromal ST2 cells and human mesenchymal stem cells by decreasing osterix expression, increasing transforming growth factor (TGF)-β expression, and suppressing endoplasmic reticulum stress proteins [28-30] (Fig. 1). Moreover, high glucose and AGEs significantly increased the expression of sclerostin in osteocyte-like MLO-Y4 cells [26]. In contrast, AGEs decreased the expression of receptor activator of nuclear factor κB ligand (RANKL), which stimulates osteoclast differentiation and activity. Furthermore, AGEs induced apoptosis of osteoblasts and osteocytes [26,28]. Taken together, these findings suggest that hyperglycemia and AGEs coordinate to low bone remodeling by decreasing RANKL expression in osteocytes.

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, lifestyle-related diseases such as diabetes, as well as vitamin B12 and folate insufficiency. Previous studies have shown that hyperhomocysteinemia increases the risk of osteoporotic fracture independently of BMD [31,32], suggesting that the deterioration of bone quality may be a dominant cause of Hcy-induced bone fragility. Li et al. previously showed that plasma Hcy levels were significantly increased in patients with T2DM than those in non-diabetes subjects, and that higher plasma Hcy levels were associated with the incidence of vertebral fractures and hip fractures in patients with T2DM [33]. Although the mechanism underlying Hcy-induced bone fragility in DM is still unclear, several studies about the effects of Hcy on osteoblast function and collagen cross-links were reported [5,34]. Diet-induced hyperhomocysteinemia decreased bone quality in vivo [34], and Hcy directly affected osteoblast lineage cells such as bone marrow stromal cells and osteoblasts. We recently showed that Hcy induced the apoptosis of osteoblastic cells by increasing oxidative stress [5]. 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 viability and function of osteoblasts as well as deteriorate bone stiffness by inhibiting formation of enzymatic collagen cross-links and increasing extracellular non-enzymatic pentosidine cross-links (Fig. 2). Moreover, we previously showed that Hcy increased oxidative stress and induced apoptosis of osteocytes by increasing NADPH oxidase 1 (Nox1) and Nox2 expressions [14,35]. These finding suggest that Hcy induces osteoblast dysfunction and AGEs accumulation and increases the apoptosis of osteocyte, resulting in decreased bone formation and bone remodeling as well as increasing AGEs collagen cross-links. Therefore, not only AGEs but also hyperhomocysteinemia may be involved in the bone fragility in patients with DM.

3. Insulin and insulin-like growth factor-I

Insulin and its signaling pathway are important for osteoblastic differentiation, collagen synthesis, and
bone formation. Patients with T1DM have a significant reduction in BMD with decreased bone formation and an increased risk of fragility fractures [36-38]. The clinical feature suggests that insulin signal is required for bone formation and bone development. Previous studies have demonstrated that osteoblasts have a functional insulin receptor and that insulin treatment stimulates the proliferation and differentiation of osteoblasts [39,40]. Moreover, osteoblast-specific insulin receptor knockout (Ob-IR−/−) mice showed reduced bone volume due to decreased bone formation and deficient number of osteoblasts [6,41]. Alkaline phosphatase (ALP) activity and osteocalcin expression were significantly decreased by inhibiting a Runx2 inhibitor, Twist2, in osteoblasts from Ob-IR−/− mice [41]. Insulin-like growth factor (IGF)-I is also known to have an anabolic effect on bone. IGF-I is expressed in osteoblasts and promotes osteoblastic differentiation and bone remodeling by autocrine and paracrine pathways in the microenvironment. Circulating IGF-I is mainly produced in the liver by growth hormone and diet, acts in an endocrine manner on bone. Previous studies showed that osteoblast-specific knockout mice of IGF-I receptor had a significant reduction in bone mass and deficient mineralization were observed [42], and that liver-specific IGF-I gene-null mice had a marked reduction in bone volume, periosteal circumference, and medial lateral width [43]. In addition, several laboratory studies have shown that the stimulatory actions of IGF-I on osteoblasts are inhibited by high glucose and AGEs [44,45], and that high glucose significantly impairs the proliferative and functional responses of osteoblastic cells to IGF-I [44]. AGEs also significantly decreased IGF-I secretion in osteoblasts [45]. Thus, hyperglycemia and AGEs may cause the resistance of osteoblasts to IGF-I actions in local environment. Therefore, IGF-I signaling is important for maintaining bone mass and strength in diabetic patients, and decreased IGF-I levels may be involved in the DM-induced bone fragility. Indeed, we have previously shown that serum IGF-I level was positively associated with serum osteocalcin levels, and inversely with the number of prevalent vertebral fractures in postmenopausal women with T2DM [8,46].

4. Adiponectin

Previous studies have shown that adipose tissue is associated with bone metabolism. Adipocytes are known to not only be an energy-storing organ but also secret a variety of biological active molecules, which are named adipokines. It has been shown that osteoblast has an adiponectin receptor, and that the adiponectin signaling stimulates proliferation, differentiation and mineralization of osteoblasts [11,47]. Previously, Luo et al. showed that recombinant adiponectin increased ALP
activity and osteocalcin expression in human osteoblasts [47]. Furthermore, we demonstrated that adiponectin activated AMPK and stimulated the differentiation and mineralization of osteoblasts by increasing bone morphogenetic protein-2 (BMP-2), and that a knockdown of adiponectin receptor by using siRNA induced an inhibition of ALP activity as well as of osteocalcin expression in osteoblastic MC3T3-E1 cells [11]. Moreover, it is also reported that adiponectin stimulated osteoclast activity by increasing RANKL expression and decreasing OPG expression in osteoblasts although adiponectin had no direct effects on osteoclasts [48]. These findings suggest that adiponectin plays important roles in bone formation and remodeling, and that hypoadiponectinemia, which is frequently seen in obese and diabetes patients, may be involved in DM-induced bone fragility. However, the association between adiponectin and bone metabolism are still unclear in in vivo and clinical studies. Several studies showed that serum adiponectin levels were positively correlated with bone formation markers [9,49], whereas a few studies showed that higher serum adiponectin were associated with the risk of fracture [9,50]. Therefore, further studies are needed to clarify the effects of adiponectin on bone metabolism and fracture risk in patients with DM.

**The association between osteocalcin and glucose metabolism**

Recently, bone is recognized as an endocrine organ regulating glucose metabolism. Previous studies using gene mutant mice models have shown that osteocalcin secreted from bone regulates whole body glucose homeostasis and that undercarboxylated osteocalcin may be its active form for the endocrine action. Lee et al. for the first time showed the roles of osteocalcin in glucose metabolism by using osteocalcin knockout (Ocn⁻/⁻) [10]. Ocn⁻/⁻ mice showed hyperglycemia and glucose intolerance, decreased β-cell and insulin secretion, decreased insulin sensitivity and adiponectin expression, and increased fat mass and serum triglyceride level. Namely, the phenotype of Ocn⁻/⁻ mice is similar to that of T2DM. In addition, recombinant osteocalcin injection improved the glucose intolerance and increased insulin expression in β-cells. Moreover, in vitro experiments showed that undercarboxylated osteocalcin significantly stimulated the expression of cyclin D1 and insulin in islets as well as of adiponectin in adipocytes, whereas carboxylated osteocalcin showed no effects on them.

Basic experimental studies described above have shown that osteocalcin plays crucial roles in glucose metabolism. However, it is important to clarify the effects of osteocalcin on glucose metabolism in humans, because the size and some amino acids of osteocalcin are different between mice and humans and osteocalcin is encoded by a single gene in humans that is highly conserved across species, while mice contain a cluster of three osteocalcin genes [51]. Kindblom et al. previously showed that total osteocalcin levels were inversely correlated with plasma glucose levels and fat mass in elderly non-diabetic subjects [52]. Fernandez-Real et al. also demonstrated that total osteocalcin levels were associated with insulin sensitivity in non-diabetes subjects [53]. Pittas et al. reported cross-sectional and longitudinal studies showing that total osteocalcin levels were inversely associated with fasting plasma glucose, fasting insulin, HOMA-IR, and fat mass in a cross-sectional analysis, and that total osteocalcin levels were associated with changes in fasting plasma glucose in a prospective analysis [54]. We also previously reported clinical studies using T2DM patients showing that total osteocalcin levels were inversely associated with glucose and visceral fat mass and positively with serum adiponectin levels, parameters of insulin secretion and its sensitivity [55,56]. In addition, we reported a longitudinal study showing that changes in osteocalcin was negatively correlated with changes in HbA1c during treatments of type 2 diabetes [57]. To examine the association of undercarboxylated osteocalcin with glucose metabolism, we previously measured serum undercarboxylated osteocalcin by using electrochemiluminescence immunoassay and analyzed the association between serum undercarboxylated osteocalcin levels and parameters of glucose metabolism in patients with T2DM. We firstly reported that serum undercarboxylated osteocalcin levels were negatively correlated with % trunk fat and visceral/subcutaneous fat ratio as well as fasting plasma glucose and HbA1c independent of various confounding factors [58]. However, the correlations of undercarboxylated osteocalcin with the parameters were almost same as those of total osteocalcin. Hwang et al. also reported that elevated serum levels of both carboxylated and undercarboxylated osteocalcin were associated with improved glucose tolerance and that undercarboxylated osteocalcin was associated with enhanced β-cell function, and that carboxylated osteocalcin was associated with improved insulin sensitivity in middle-age male healthy subjects [59]. On the contrary, Iki et al. showed
that undercarboxylated osteocalcin levels were significantly and inversely correlated with fasting plasma glucose, HbA1c and HOMA-IR after adjusting for total osteocalcin, while total osteocalcin was not associated with these parameters after adjusting for undercarboxylated osteocalcin [60]. Thus, these findings suggest that osteocalcin may be involved in glucose metabolism not only in rodents but also in humans. However, because it is still controversial whether undercarboxylated osteocalcin is the active form as an endocrine factor in humans, further large-scale studies and meta-analysis are necessary in future.

**The roles of AMPK in bone metabolism and the interaction between bone and glucose metabolism**

AMPK is known to be a crucial regulator of energy and metabolic homeostasis at the cellular and whole-organism levels [61,62]. An increase in the cellular AMP/ATP ratio activates AMPK through the phosphorylation of α subunit. Once activated, AMPK inactivates several metabolic enzymes involved in ATP-consuming cellular events, including cholesterol and protein synthesis [63]. Accumulating evidence has shown that AMPK is a candidate therapeutic target for metabolic disease, atherosclerosis, and cancer [64,65]. AMPK subunits are expressed in bone tissue and cells, and the AMPKα1 subunit is the dominant catalytic isoform expressed in bone [66,67]. Previous studies have demonstrated that the AMPK signaling plays roles in bone physiology [68]. We previously reported that AMPK activation stimulated the differentiation and mineralization of osteoblasts by enhancing the expression of BMP-2 and endothelial nitric oxide synthase via the inhibition of mevalonate pathway [11-13]. Moreover, other researchers also reported that AMPK activation induced calcified nodule formation in primary osteoblasts, whereas AMPK inhibition suppressed the effects of AMPK and osteoblastic differentiation [69,70]. On the other hand, several studies showed that AMPK plays roles in osteoclastogenesis. AMPK activation directly inhibited osteoclastogenesis [71,72] and indirectly suppressed osteoclast differentiation by stimulating OPG and reducing RANKL expression in osteoblasts [73]. Moreover, we previously showed that AMPK activation significantly decreased RANKL expression and increased sclerostin expression in osteocyte MLO-Y4 cells, and that knockdown of AMPKα1 significantly increased the RANKL expression [74]. Taken together, these findings suggest that AMPK activation in osteoblasts stimulates their differentiation and mineralization, but AMPK activation in osteocytes suppresses the differentiation of osteoblasts by increasing sclerostin expression, maybe as a feedback loop. In contrast, AMPK activation in osteoblasts and osteocytes inhibits the differentiation of osteoclasts by decreasing RANKL expression and stimulating OPG expression, and AMPK activation in osteoclasts suppresses their differentiation and activity. Indeed, a few studies using genetic mutant mice have shown that inactivation of AMPK decreases bone mass in vivo [65,68,74]. A conventional knockout mice of AMPKα subunit showed that both cortical and trabecular bone compartments were significantly smaller in the AMPK knockout mice compared to the controls due to increased bone resorption [74].

As previously described, osteoblasts have a functional adiponectin receptor and adiponectin increases osteoblastic differentiation and bone remodeling through AMPK signaling. Also, adiponectin and AMPK activation stimulate osteocalcin expression in osteoblasts [11,12]. Adiponectin stimulates osteocalcin expression in osteoblasts and the differentiation of osteoclasts, and osteocalcin alternatively stimulates the expression of adiponectin in adipocytes; therefore, it is reasonable to assume that there is an endocrine loop between bone and adipose tissue through AMPK activation in bone. On the other hand, it is reported that IGF-I modulates AMPK activity in various cell types [75]. Furthermore, a recent study demonstrated that AMPK activation is required for the activation of IGF-I in early stages of osteoblast differentiation [76]. Therefore, AMPK may play pivotal roles as a key molecule in IGF-I-regulated bone modeling and remodeling although the roles of osteoblast AMPK in IGF-I signaling are still unclear. In addition, several studies showed that osteocalcin affect GH-IGF-I axis and liver function. It is demonstrated that osteocalcin injection significantly increased growth hormone secretion from pituitary, and hepatic expression of GH receptor and IGF-1 [77]. Furthermore, osteocalcin injection improved high fat-induced glucose intolerance, insulin resistance, and hepatic steatosis in vivo [78]. Therefore, the IGF-I-AMPK signal in bone may be involved in the interaction between bone and liver.

Metformin, an anti-diabetic drug, is worldwide used for the treatment of T2DM. Metformin is known to enhance the sensitivity for insulin in several tissues
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such as liver and muscle via AMPK activation, resulting in increasing insulin action and lowering blood glucose levels. We have previously shown that metformin stimulated the differentiation and mineralization of osteoblastic cells and osteocalcin expression by activating AMPK [13]. In addition, other researchers have demonstrated that metformin inhibits RANKL expression and stimulates OPG expression in osteoblasts; supernatants from cultured osteoblasts treated with metformin significantly suppressed osteoclast formation and expression of tartrate-resistant acid phosphatase and cathepsin K in osteoclasts [72], leading to an increase in bone mass. Indeed, several clinical studies suggested the beneficial effects of metformin on fracture risk in patients with T2DM [79,80]. Thus, the results of these studies may be understood to show the role of AMPK as the target molecule of metformin in bone, and suggest that AMPK activation may be a candidate for the treatment of DM-induced bone fragility (Fig. 3).

Conclusion

Emerging evidence from epidemiological, clinical, and experimental studies has shown that DM induces bone fragility mainly due to accumulation of AGEs collagen cross-links in bone and dysfunctions of osteoblast and osteocyte with low bone remodeling. Osteoblast and osteocyte dysfunctions may be caused by complex diabetic condition such as hyperglycemia, high AGEs and Hcy levels in the circulation, decreased insulin and IGF-I action, and low adiponectin levels. Moreover, bone interacts with glucose metabolism by regulated insulin secretion from pancreas as well as adipokines from adipose tissue, and that bone is an active tissue involved in energy homeostasis. Although several clinical studies suggested that osteocalcin might be involved in systemic glucose homeostasis, there are no direct evidence that osteocalcin regulates glucose metabolism in human. Previous studies reported that adiponectin, IGF-I, and metformin stimulate osteoblastic differentiation partly through AMPK activation. Thus, AMPK is a candidate for a therapeutic target for DM-induced bone fragility. However, this relatively new topic should be further explored to understand the pathophysiology of DM-induced bone fragility and develop a new therapy of DM.

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