

Derangement of calcium metabolism in diabetes mellitus: negative outcome from the synergy between impaired bone turnover and intestinal calcium absorption

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Abstract Both types 1 and 2 diabetes mellitus (T1DM and T2DM) are associated with profound deterioration of calcium and bone metabolism, partly from impaired intestinal calcium absorption, leading to a reduction in calcium uptake into the body. T1DM is associated with low bone mineral density (BMD) and osteoporosis, whereas the skeletal changes in T2DM are variable, ranging from normal to increased and to decreased BMD. However, both types of DM eventually compromise bone quality through production of advanced glycation end products and misalignment of collagen fibrils (so-called matrix failure), thereby culminating in a reduction of bone strength. The underlying cellular mechanisms (cellular failure) are related to suppression of osteoblast-induced bone formation and bone calcium accretion, as well as to enhancement of osteoclast-induced bone resorption. Several other T2DM-related pathophysiological changes, e.g., osteoblast insulin resistance, impaired productions of osteogenic growth factors (particularly insulin-like growth factor 1 and bone morphogenetic proteins), overproduction of pro-inflammatory cytokines, hyperglycemia, and dyslipidemia, also aggravate diabetic osteopathy. In the kidney, DM and the resultant hyperglycemia lead to calciuresis and hypercalciuria in both humans and rodents. Furthermore, DM causes deranged functions of endocrine factors related to mineral metabolism, e.g., parathyroid hormone, 1,25-

dihydroxyvitamin D₃, and fibroblast growth factor-23. Despite the wealth of information regarding impaired bone remodeling in DM, the long-lasting effects of DM on calcium metabolism in young growing individuals, pregnant women, and neonates born to women with gestational DM have received scant attention, and their underlying mechanisms are almost unknown and worth exploring.

Keywords Bone loss · Calcium wasting · Growth plate · T1DM · T2DM · Osteoporosis

Introduction

Diabetes mellitus (DM) is a worldwide metabolic disease characterized by chronic hyperglycemia, which results from insufficient insulin secretion and/or insulin resistance in target tissues or both [1, 2]. Chronic hyperglycemia leads to macrovascular and microvascular complications in various organs, e.g., heart, brain, kidney, eyes, and bone, the last of which culminates in the so-called diabetic osteopathy [1, 2]. In other words, DM not only disturbs normal functions of vital organs (for reviews, see [3–5]) but also impairs calcium metabolism by disrupting the functions of calcium-regulating organs, namely the intestine, bone, and kidney. The present article thus focuses on the underlying mechanisms of the negative effects of DM on calcium homeostasis as well as their long-term consequences.

Overview of DM and insulin resistance

Generally, DM is classified into 4 types based on defects in the mechanism of insulin action, that is, type 1 DM (T1DM), type 2 DM (T2DM), gestational DM (GDM) and

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other specific types of DM, the last of which includes genetic defects in insulin processing, insulin action, or β -cell function [1, 2]. T1DM, or insulin-dependent DM, accounts for 5–10 % of diabetic patients. It is caused by autoimmune destruction of β -cells in the islet of Langerhans, resulting in reduced β -cell mass and inadequate insulin secretion. Autoimmunity in T1DM comes in diverse forms, such as islet autoantibodies, insulin autoantibodies, autoantibodies to tyrosine phosphatase IA-2 and IA-2 β and glutamic acid decarboxylase isoform-65 (GAD65), the last of which is usually detected in the early hyperglycemic stage of T1DM [1, 2]. T1DM commonly takes effect in childhood and adolescence, but it can occur at any age up to old age [1].

The more common type is T2DM or non-insulin-dependent DM, which accounts for 90–95 % of diabetic patients. It results from insulin resistance and relative insulin deficiency rather than absolute deficiency. Most T2DM patients have obesity or high body fat distribution that often occurs with insulin resistance in the target tissues, such as skeletal muscle and adipose tissue. This form of DM is more strongly correlated with genetic predisposition than immune-mediated T1DM. Because of insulin resistance, a compensatory increase in insulin secretion is required to maintain normal plasma glucose levels [6, 7]. A number of post-receptor defects have been proposed to explain the pathogenesis of insulin resistance, such as defects in insulin-regulated phosphorylation/dephosphorylation [7]. For example, DM causes a phosphatidylinositol-3-kinase (PI3K) signaling defect that may reduce translocation of glucose transporters (e.g., GLUT4) to the plasma membrane, thereby impairing glucose uptake [6, 8]. Other abnormalities include the accumulation of ectopic lipid metabolites that later impair mitochondrial oxidative phosphorylation and reduce insulin-stimulated mitochondrial ATP production, and also generate reactive oxygen species (ROS) [6–8]. Lipid metabolite-mediated insulin resistance results from the accumulation of diacylglycerol (DAG), which, in turn, activates protein kinase C isoform θ (PKC θ) and subsequently impairs the translocation of GLUT4-containing storage vesicles to the plasma membrane [6, 8]. Of note, inflammatory mediators secreted from adipocytes also contribute to insulin resistance, such as tumor necrosis factor (TNF)- α , and interleukin (IL)-6, and suppressor of cytokine signaling (SOCS)-1 and -3 [6], some of which, particularly TNF- α , and IL-6, are also potent stimulators of osteoclastogenesis and bone resorption [9].

Regarding GDM, it is a condition in which women without previously diagnosed DM show hyperglycemia during pregnancy or persisting after delivery [1]. GDM women with hypertensive disorder, preterm delivery, and multiparity have increased risk of developing T2DM in

their lifetime [10]. GDM is also more prevalent in women of certain ethnic groups, e.g., Hispanic, and Native American [11]. Interestingly, a clinical study investigating the risk factors associated with development of postpartum T2DM in 843 GDM women found that high pre-pregnancy body mass index (BMI) was an important risk factor for both early and late T2DM conversions [12]. Indeed, pregnancy places a stress on maternal calcium metabolism, especially in the third trimester, during which \sim 200 mg elemental calcium per day is transferred across the placenta for fetal osteogenesis (for reviews, see [13, 14]). However, bone changes in GDM mothers have received little attention.

As for other specific types of DM, the etiologies are diverse, but most of them are genetic diseases or consequences of exocrine pancreatic disorders and endocrinopathies [1]. The most common genetic defect of β -cell is maturity-onset diabetes of the young (MODY), which is characterized by the onset of hyperglycemia at an early age (generally before 25 years), and impairment of insulin secretion with minimal or no defect in insulin action [1]. MODY is a monogenic disorder that inherits in an autosomal dominant pattern. Approximately 1 % of MODY patients are misdiagnosed as T1DM or T2DM [1, 15]. The most common forms of MODY are associated with mutations of hepatocyte nuclear factor (HNF)-1 α and glucokinase genes, which account for 80 % of all MODY cases [1, 15]. HNF-1 α is a transcription factor directly involved in pancreatic development and β -cell differentiation [16]. Glucokinase serves as glucose detector for β -cells, and is responsible for the conversion of glucose to glucose-6-phosphate, which in turn stimulates insulin secretion [1]. The defect of the glucokinase gene thus directly affects β -cells secretion of insulin.

Body calcium homeostasis

Calcium is an important element for several biological processes, such as cell division and growth, blood coagulation, cardiovascular homeostasis, hormone responses, neural electrical activity, and bone formation [17, 18]. Ninety-nine percent of calcium is stored in bone, while the remaining one percent is distributed in the intracellular fluid (ICF) and extracellular fluid (ECF). The total plasma calcium and ionized calcium must be maintained within a narrow range (8.5–10.5 mg/dL and 4.65–5.25 mg/dL, respectively) [18]. Because a large change of plasma calcium concentration, either a decrease (hypocalcemia) or increase (hypercalcemia), is lethal, therefore, calcium level is tightly regulated by three classical calcium-regulating hormones, namely, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], and calcitonin, as well

as other endocrine and paracrine factors [e.g., estrogen, androgens, adrenal glucocorticoids, prolactin, insulin-like growth factor (IGF)-1, and fibroblast growth factor (FGF)-23]. Not only the plasma calcium level, but the whole body calcium metabolism is also controlled by these calcium-regulating hormones to ensure adequate intestinal calcium absorption, proper storage of calcium in bone, and excretion of excess calcium via the kidney.

To maintain normal calcium homeostasis, two aspects of calcium metabolism have to be considered. The first aspect is the total amount of calcium in the body that depends on the balance between calcium absorbed by the intestine and that excreted by the kidney. In normal adults with daily calcium intake of 1000 mg, ~200 mg (~20 %) is absorbed by the intestine, while ~800 and ~200 mg are excreted in feces and urine, respectively [17]. Thus, calcium input balances total calcium output. The second aspect is the regulation of the plasma-ionized calcium within a normal range of 4.65–5.25 mg/dL. Specifically, hypocalcemia increases the circulating levels of parathyroid hormone (PTH) and $1,25(\text{OH})_2\text{D}_3$ levels. PTH stimulates calcium reabsorption and suppresses phosphate reabsorption in the renal tubules, and also enhances the conversion of $25(\text{OH})\text{D}_3$ into $1,25(\text{OH})_2\text{D}_3$, which, in turn, increases the intestinal calcium absorption. PTH also stimulates bone resorption to release calcium into the circulation [17].

In contrast, when hypercalcemia occurs, negative feedback mechanisms limit calcium entry and increase calcium excretion and the normal level of plasma ionized calcium is restored [17]. The feedback mechanism involves calcium-sensing receptors (CaSR) in the parathyroid chief cells that sense elevated ionized calcium level, thus inhibiting PTH release and $1,25(\text{OH})_2\text{D}_3$ synthesis [17]. Recently, FGF-23 has been added to the list of calcium regulatory hormones as it can inhibit $1,25(\text{OH})_2\text{D}_3$ -induced intestinal calcium absorption [19, 20]. Therefore, the intestinal calcium absorption is inhibited directly by reduction in $1,25(\text{OH})_2\text{D}_3$ production, and indirectly by increased FGF-23 production from the osteocytes in bone or from local production in the intestine [17, 19, 21].

In the diabetic condition, derangement of calcium homeostasis is multifactorial and, although it was reported in 1979 that diabetic patients exhibited normal calcium homeostasis, i.e., no change in plasma levels of total and ionized calcium, PTH, $25(\text{OH})\text{D}_3$, and $1,25(\text{OH})_2\text{D}_3$ [22], new investigations in the 2000s mostly found impaired calcium homeostasis in DM and associations between calcium-regulating hormones and the development of DM [23, 24]. For example, individuals with hypovitaminosis D had a higher risk of developing insulin resistance, metabolic syndrome, and incidence of T2DM [23, 24]. Recently, there was a report that a high level of PTH was

associated with DM, and this association varied according to ethnic groups—i.e., higher incidence in Caucasians [25]. Besides the calcium-regulating hormones, DM also affects the function of calcium-regulating organs, causing impaired intestinal calcium absorption, bone deterioration, decelerated bone elongation and renal calcium wasting (Fig. 1); the details are discussed below.

DM-associated impairment of intestinal calcium absorption

Unlike bone, intestinal calcium absorption, which is the only route for calcium entry into the body, is rarely investigated in DM. Most intestinal calcium absorption studies have been performed in alloxan- or streptozotocin (STZ)-induced T1DM animal models that showed lower rates in DM [26–29]. In alloxan-induced DM rats, the decrease in net calcium absorption was due to a decrease in the lumen-to-plasma calcium flux in the duodenum and ileum [27]. This reduction in calcium absorption could be restored to control level by insulin [28], confirming the association between DM and intestinal impaired calcium absorption.

At present, it is not known whether the reduction in intestinal calcium absorption results directly from insulin deficiency or from other hormonal disturbance. Several studies in diabetic rats reported that the reduction in intestinal calcium absorption occurred concurrently with decreases in the circulating $1,25(\text{OH})_2\text{D}_3$ level, intracellular vitamin D receptor number, and cytoplasmic calcium-binding protein calbindin- D_{9k} in the enterocytes [29, 30]. However, other investigators have reported a compensatory upregulation of transcellular and paracellular calcium transporter expression in the intestinal epithelial cells of diabetic animals. As demonstrated by Rivoira and co-workers [26], the expressions of transient receptor potential vanilloid family calcium channel-6 (TRPV6), plasma membrane calcium-ATPase-1b (PMCA_{1b}), sodium-calcium exchanger-1 (NCX1), and claudin-2 were significantly increased in the intestinal epithelium of STZ-induced T1DM rats. They also found that DM was accompanied by increased oxidative stress and production of free radicals (e.g., superoxide anion), which could alter duodenal permeability in STZ-induced diabetic rats [26]. However, whether T2DM affects intestinal calcium absorption and intestinal permeability in humans remains to be investigated.

Diabetic osteopathy

T1DM and T2DM have been reported to aggravate osteopenia and osteoporosis that increase fracture risk [31, 32], but pathological bone changes in T1DM and

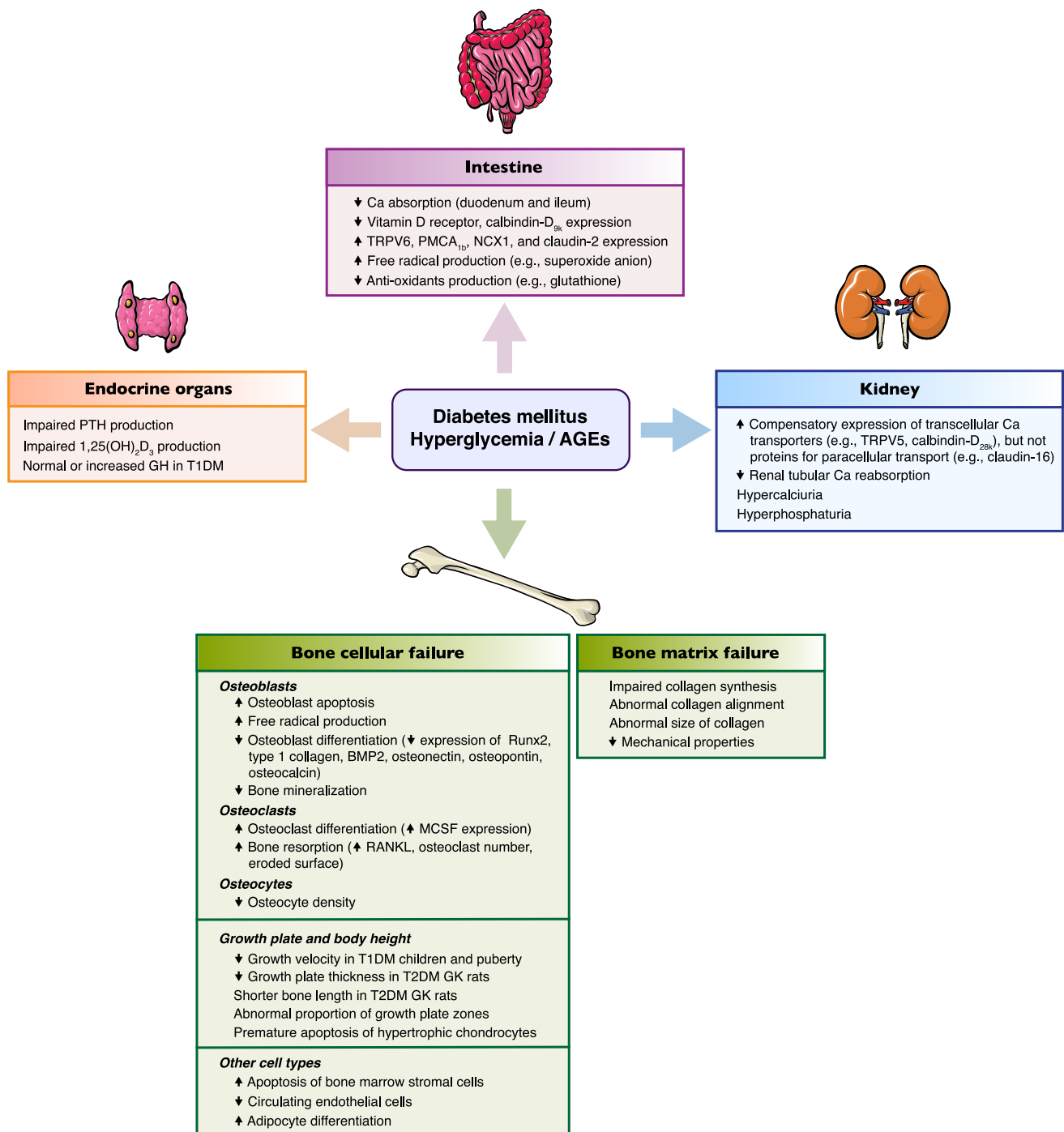


Fig. 1 Possible deleterious effects of diabetes mellitus, hyperglycemia and advanced glycation end products (AGEs) on metabolism of calcium-regulating hormones and calcium-regulating organs, i.e., intestine, kidney, and bone

T2DM are markedly different in terms of cellular and molecular mechanisms [33–37]. Low bone mass is consistently observed in T1DM patients [33–35]. However, for T2DM, there have been reports of both high and low bone mineral density (BMD) [36–39]. Previously, it was thought that the high bone mass in T2DM might be an adaptation to sustain high body weight and might postpone osteoporosis

[37, 39]. More advanced high-resolution, peripheral quantitative computed tomography (HR-pQCT) or microindentation techniques have supported the notion that T2DM patients indeed have deteriorated bone functions, such as impaired bone microstructure and mechanical properties, and abnormal function of bone cells [38, 40, 41]. Although evidence is scant, a few studies on GDM have reported

Table 1 Cytokines with osteoclastogenic activities in DM

Cytokines	Major findings	Refs.
RANKL	Elevated in plasma of T1DM children	[101]
	Elevated in bone marrow of polygenic TallyHo/JngJ T2DM mice	[102]
	Increased mRNA expression in STZ-treated T1DM mice	[48]
IL-1	Elevated in serum during early onset of T1DM in STZ-treated mice	[103]
IL-6	Elevated in blood and bone marrow of TallyHo/JngJ T2DM mice	[102]
	Elevated in serum during early onset of T1DM in STZ-treated mice	[103]
TNF- α	Elevated in blood and bone marrow of TallyHo/JngJ T2DM mice	[102]
	Increased mRNA expression in STZ-treated T1DM mice	[48]
IFN- γ	Elevated in blood and bone marrow of TallyHo/JngJ T2DM mice	[102]
	Elevated in serum during early onset of T1DM in STZ-treated mice	[103]
MCSF	Increased mRNA expression in STZ-treated T1DM mice	[48]
MCP-1	Elevated in serum during early onset of T1DM in STZ-treated mice	[103]

RANKL receptor activator of nuclear factor- κ B ligand, *IL-1* interleukin-1, *IL-6* interleukin-6, *TNF- α* tumor necrosis factor- α , *IFN- γ* interferon- γ , *MCSF* macrophage colony-stimulating factor, *MCP-1* monocyte chemoattractant protein-1, *STZ* streptozotocin

greater reduction in maternal BMD and bone loss when compared with non-diabetic mothers [42].

Regardless of the types of DM, pathogenesis of DM-induced osteoporosis can result from pathophysiological changes in bone compositions, i.e., cellular failure and extracellular matrix failure, both of which are provoked by factors such as insulin resistance, advanced glycation end products (AGEs) and ROS. Regarding the cellular failure, DM is correlated with abnormal or diminished activities of bone cells (i.e., osteoblasts, osteoclasts and osteocytes) as well as neighboring cells in bone, such as adipocytes, mesenchymal cells, and endothelial progenitor cells [38, 43, 44]. Several in vitro investigations reported that high blood glucose induced ROS production in osteoblasts, which, in turn, promoted osteoblast apoptosis, and suppressed osteoblast differentiation and mineralization as indicated by decreased expressions of osteoblast differentiation markers, i.e., *runx*-related transcription factor-2 (*Runx2*), type I collagen, osteonectin and osteocalcin [43]. In addition, ROS enhanced transformation from osteoblast lineage to adipocyte lineage by increasing the expression of adipogenic markers, i.e., peroxisome proliferator-activated receptor (PPAR)- γ , adipocyte fatty acid binding protein (aP2), resistin and adiponin [45]. The final outcome is a reduction in the mature osteoblast numbers and thus bone formation [43, 45, 46]. Consistently, in vivo investigation in insulin-resistant high-fat diet-fed Zucker diabetic fatty (ZDF) rats also showed downregulation of osteoblast differentiation markers, namely *Runx2*, bone morphogenetic protein (BMP-2), osteocalcin, and osteopontin [47]. In Goto-Kakizaki (GK) T2DM rats, bone histomorphometric analysis confirmed that DM reduced osteoblast activities (e.g., osteoblast surface, mineralizing surface and bone formation rate), while increasing osteoclast bone resorption

(e.g., osteoclast surface and eroded surface) [38]. This accelerated bone resorption resulted from an increase in osteoclast numbers and expression of osteoclastogenic mediators, e.g., *TNF- α* , macrophage colony-stimulating factor (MCSF), receptor activator of nuclear factor- κ B ligand (RANKL) and vascular endothelial growth factor (VEGF)-A [48]. The DM-associated bone loss is consistent with increases in mRNA and circulating levels of osteoclastogenic cytokines, as summarized in Table 1.

Osteocytes—the most abundant bone cells entombed in the bone matrix—are also affected, as indicated by a reduction in osteocyte density (number of osteocyte-occupied lacunae per unit area) in diabetic rats [44]. Since osteocytes are mechanosensitive cells that play an important role in bone response to mechanical stimuli, DM may reduce the mechanical signal transduction for bone remodeling and may indirectly weaken the collagen alignment with increased fracture risk [44, 49, 50].

Besides bone cells, a number of other cell types in bone, such as bone marrow stromal cells (BMSCs) or endothelial progenitor cells, are also decreased in diabetic condition. The underlying mechanism—at least in rats—probably related to AGEs-induced apoptosis of BMSCs through *TNF- α* production, oxidative stress, and finally caspase activation [51]. The number of circulating endothelial cells are apparently decreased in diabetic mice, causing delay in angiogenesis, and eventually lead to poor bone quality, poor healing at fracture sites, and increased fracture risk [52].

Regarding the bone extracellular matrix failure, DM was recently reported to alter the structure of type I collagen causing impaired bone strength [53]. Normally, collagen synthesis takes place both intracellularly and extracellularly. Initially, it is synthesized as procollagen, which is

exported from the cell in the form of procollagen. Upon secretion, procollagen undergoes three steps of transformation in the extracellular space, i.e., (1) enzymatic removal of the free endings by procollagen peptidase to give rise to tropocollagen, (2) cross-linking of tropocollagen molecules to form collagen fibrils by lysyl oxidase, and (3) side-by-side cross-linking of collagen fibrils to form collagen fibers [54]. Large amounts of AGEs and ROS commonly accumulated in diabetic condition contribute to bone deterioration by impairing collagen synthesis and collagen alignment [45, 50, 55]. AGEs are heterogeneous groups of molecules formed from the non-enzymatic reaction between reducing sugars and free amino groups of proteins, lipids, and nucleic acids. They can form cross-links between proteins, and, in doing so, alter structure and function of those proteins in extracellular matrix, basement membrane, and blood vessel wall. AGEs are often found in the plasma proteins of patients with DM and renal failure [50, 56]. They can suppress the production of phosphoprotein-1 (the mature osteoblast marker) and lysyl oxidase, the latter being essential for collagen crosslink [50]. Recently, by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), abnormally large collagen fibrils and aberrant collagen fibril alignment were found in rats with AGEs accumulation induced by adenine treatment [50]. These animals exhibited low bone quality as evidenced by a reduction in mechanical bone strength, i.e., rigidity, yield moment, ultimate moment, and energy to fracture [57, 58]. In addition to AGEs, ROS produced by mitochondrial dysfunction (such as hydrogen peroxide), malondialdehyde, and cytosolic ROS also impaired osteoblastogenesis, thereby preventing type I collagen production and compromising bone quality [45, 55].

Growth retardation in DM

While DM-induced osteopathy has been extensively investigated, impaired longitudinal bone growth, especially during childhood and adolescence, another feature of bone defects associated with T1DM, T2DM, and other congenital DM, is largely unknown. Normally longitudinal bone growth is under the influence of both local and systemic factors, e.g., growth hormone (GH), insulin-like growth factor-1 (IGF-1) and insulin [59, 60]. GH from the anterior pituitary stimulates longitudinal bone growth specifically via indirect and direct actions. The indirect action is mediated by IGF-1 from the liver, which increases growth plate chondrocyte proliferation [59, 61]. GH and insulin also play important roles in longitudinal bone growth by increasing proliferation of the growth plate chondrocytes and expression of endochondral bone formation related

genes [62, 63]. Thus, insulin deficiency in T1DM ultimately impairs growth plate development and also longitudinal bone growth. Again, there have been reports of both normal and impaired growth in DM subjects [64–66].

Growth retardation can occur after the onset of DM [64, 66]. Lebl et al. [64] reported longitudinal change of height in 587 diabetic children and teenagers (~0.3–20 years of age). They found that the heights of male and female T1DM in the first observation were higher than the mean standard, but subsequently declined. Consistently, Muñoz and co-workers [66] found that the heights of T1DM patients in different pubertal stages were within normal range, but the growth velocity was slightly below average. The reason why the height of T1DM patients was normal during diabetic onset but decreased later remains unclear, but it might link to synchronization between diabetic onset and pubertal growth spurt or partial GH resistance in T1DM patients [64, 66], since circulating GH was reportedly increased in T1DM adolescents [67].

Recent investigations by Lapmanee et al. [38] and Aiemplapa et al. [68] also showed aberrant growth plate morphology and abnormal chondrocyte function in diabetic animals. Generally, bone elongation is controlled by proliferation and differentiation of chondrocytes in the growth plate. The growth plate is histologically divided into 3 zones, i.e., resting zone (RZ), proliferative zone (PZ) and hypertrophic zone (HZ). The RZ contains small chondroblasts with low mitotic activity embedded in extracellular matrix. Chondroblasts gradually migrate to the PZ where cells become highly proliferative and align into parallel vertical columns. The proliferative chondrocytes enlarge and become hypertrophic cells in HZ [69]. Before undergoing apoptosis, they are replaced by osteoblasts that arrive with vascularization. The cartilaginous scaffold thus acts as a template for bone formation and bone elongation [54, 59, 69]. Apparently, strong stimuli that disrupt growth plate differentiation could have an effect on the overall growth plate morphology and the extent of longitudinal bone growth. Since MODY develops during childhood or adolescence, it could profoundly affect chondrocyte function and endochondral bone growth. Even though short stature is not an important clinical manifestation of MODY, its negative effect on the growth plate is likely to result in poor-quality bone tissues.

Laboratory investigations of the growth plate of diabetic animals mostly show growth arrest, reduction of growth plate height or abnormal proportion of growth plate zones [38, 68, 70, 71]. For example, STZ-induced T1DM rats showed growth arrest with a marked reduction of the growth plate height [70]. Consistent with T1DM, T2DM was found to produce similar manifestations. Histological study of femur and tibia of GK rats has revealed shorter femoral length with a reduction in total growth plate and

HZ heights [38]. Furthermore, Aiemlapa et al. [68], by using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique, found that the shorter bone length in GK rats resulted from premature apoptosis of chondrocytes in HZ. Such a reduction in the number of mature chondrocytes induced compensatory overexpression of parathyroid hormone-related protein (PTHrP), vascular endothelial growth factor (VEGF) and Runx2, all of which induce chondrocytes to differentiate. Despite this body of evidence on T1DM and T2DM growth plate changes, more study is required to demonstrate the underlying cellular and molecular mechanisms that would help to understand the etiology of skeletal disorders in DM.

DM-related renal calcium wasting

Several lines of evidence have suggested that DM in both humans and rodents profoundly affects renal calcium handling, such as decreasing $1,25(\text{OH})_2\text{D}_3$ synthesis, as a consequence of prolonged hyperglycemia and dysregulation of calciotropic hormone metabolism [72–75]. An early study of glucose overload in rats showed that both oral and intravenous glucose administration induced hypercalciuria independently of urine flow rate and sodium excretion [76]. Tubular calcium reabsorption in STZ-induced diabetic rats was markedly reduced, which could be reversed by insulin administration [73]. GDM is also associated with severe hypercalciuria in rats [77]. Both urinary calcium and phosphate excretion were increased in female BioBreeding (BB) rats—an animal model of T1DM—and their neonates manifested fewer ossification centers and lower bone mineral content than neonates from healthy dams [78]. Hypercalciuria and hyperphosphaturia were also evident in T1DM children aged between 6 and 12 years [79]. However, the underlying cellular and molecular mechanisms are not well understood. Lee and co-workers [80] reported a paradoxical upregulation of transcellular calcium-transporting protein expression (e.g., TRPV5 and calbindin- $\text{D}_{28\text{k}}$), but not the paracellular protein expression (i.e., claudin-16, normally required for calcium and magnesium reabsorption in the thick ascending limb of the loop of Henle) in the renal tubules of STZ-induced diabetic rats, suggesting that this is a compensatory response to long-term renal calcium wasting. Thus, calciuria in T1DM might have resulted from the DM-induced impairment of renal calcium transporter activities rather than their protein expression.

A decrease in insulin production and release in T1DM or insulin resistance in T2DM is believed to contribute to a reduction in renal calcium (and magnesium) reabsorption. A study in isolated perfused mouse thick ascending limb showed that insulin significantly increased the

transepithelial potential difference—a driving force for passive calcium reabsorption—as well as the transepithelial calcium and magnesium transport [81]. Therefore, this driving force for calcium reabsorption is probably reduced under diabetic condition, thereby culminating in increased calcium excretion. On the other hand, amino acids that can induce insulin secretion are able to induce tubular calcium reabsorption [82]. Nevertheless, in parathyroidectomized female rats subjected to intravenous arginine infusion, the arginine-induced insulin secretion was associated with urinary calcium excretion [82]. This discrepancy can be explained by an increase in glomerular filtration rate secondary to amino acid loading, which leads to renal calcium excretion. Indeed, the stimuli or pattern of insulin secretion may also determine the final outcome of renal calcium handling.

In addition, renal calcium loss in DM can result from dysregulation of the calciotropic hormones, such as PTH and $1,25(\text{OH})_2\text{D}_3$. It has been reported that the PTH-induced stimulation of $1,25(\text{OH})_2\text{D}_3$ production was impaired in diabetic condition, whereas insulin treatment successfully rescued $1,25(\text{OH})_2\text{D}_3$ production [83]. Another evidence comes from the study of hyperinsulinemic ZDF rats (a T2DM model), in which a reduction in the renal expression of megalin and disabled (Dab)-2 proteins can cause an impaired receptor-mediated endocytosis of vitamin D-binding protein-bound $25(\text{OH})\text{D}_3$, leading to urinary loss of $25(\text{OH})\text{D}_3$ and a decrease in circulating $25(\text{OH})\text{D}_3$ level [84]. It is of interest that the bone-derived phosphaturic hormone FGF-23 probably contributes to changes in renal calcium handling during diabetic condition. FGF-23 has recently been demonstrated to be a negative regulator of intestinal calcium absorption [19–21]. Meanwhile, this hormone is also postulated to be a counter-regulatory factor that alleviates vitamin D toxicity and hypercalcemia by directly suppressing PTH release and renal $1,25(\text{OH})_2\text{D}_3$ production [85]. Moreover, the TRPV5-mediated renal calcium reabsorption is dependent on FGF-23 and its co-receptor (soluble α -Klotho) [86, 87]. The release of soluble Klotho is insulin-dependent [88]. Thus, it is tempting to speculate that the FGF-23-regulated renal calcium reabsorption, if present, is probably impaired under diabetic condition. Fajol et al. [89] recently reported that transgenic mice expressing Akt/PKB/SGK-resistant glycogen synthase kinase (GSK)-3 is characterized by hypophosphatemia, phosphaturia, and calciuria with increases in serum FGF-23 levels. Since Akt, PKB, and SGK are known to mediate intracellular insulin signaling, this study suggested an interplay between insulin and FGF-23 in the regulation of renal calcium handling.

Furthermore, FGF-23 also has direct effects on osteoblasts by enhancing the expression of osteoblast differentiation markers, e.g., Runx2, osterix, alkaline phosphatase and

osteopontin, in a dose-dependent manner [90]. They also express receptors for FGF-23 [90]. Therefore, abnormally high circulating FGF-23 in DM-associated chronic kidney disease may contribute to deterioration of the coupling process of bone remodeling and bone quality in T2DM [91].

Perspectives and conclusions

It is clear that DM, hyperglycemia and associated complications (e.g., AGEs production and microvascular damage) interfere with normal functions of the three calcium-regulating organs, namely the intestine, bone, and kidney. Moreover, they also lead to dysregulation of calciotropic hormone actions, thereby worsening the already impaired function of the calcium-regulating organs. As demonstrated in the small intestine and cultured intestinal cells, DM as well as elevated glucose concentration and AGEs diminish calcium absorption, presumably by downregulating nuclear vitamin D receptor expression and inducing oxidative stress [29, 30, 43, 45, 46]. However, how DM alters the expression of genes and proteins related to calcium transport in the small intestine, and whether calcium absorption in the large intestine is impaired, are not known. Moreover, since the proximal part of the large intestine, particularly the cecum, has the highest rate of calcium uptake compared to other intestinal segments, and since surgical removal of the cecum can cause osteopenia in rats [92–95], it is hypothesized that the DM-induced impairment of calcium transport across the large intestine would substantially contribute to impaired calcium metabolism and diabetic osteopathy.

In bone, DM induces both cellular and matrix failure in association with low BMD, especially in T1DM. Specifically, DM and hyperglycemia lead to uncoupling of bone remodeling, i.e., suppression of osteoblast function and enhancement of osteoclastogenesis and osteoclast function. These osteoblasts suffer from oxidative stress, downregulation of key protein expression (e.g., Runx2, type I collagen, and osteocalcin) and apoptosis, whereas osteoclasts become more active in resorbing bone. DM is also associated with a reduction in osteocyte density, while enhancing adipocyte differentiation. Meanwhile, hyperglycemia and overproduction of AGEs in both T1DM and T2DM impair collagen synthesis, produce abnormal collagen alignment and fibril size, and increase glycosylated collagen, thereby resulting in matrix failure and poor bone mechanical properties. Nevertheless, how other DM complications, such as microangiopathy in bone and dyslipidemia, affect bone remodeling are inconclusive. Furthermore, since the autonomic nervous system, especially the sympathetic activity through β -adrenergic receptors, has been reported to modulate bone remodeling

[96–98], it is tempting to speculate that diabetic neuropathy and the resultant dysautonomia probably contribute to bone loss in DM patients.

Although the deleterious effects of DM on bone and calcium metabolism in adult and aging individuals have been widely investigated, the effects in young growing individuals, pregnant and lactating mothers, fetuses and neonates born to mothers with GDM have received much less attention. Available data suggest that DM in the young impairs longitudinal bone growth by inducing premature hypertrophic chondrocyte apoptosis, and early vascular invasion into the epiphyseal chondro-osseous junction [38, 68]. However, the circulating profiles of growth factors, which are important for the epiphyseal function and elongation of long bones, remain unknown. In addition, how DM alters local production of chondroregulatory factors, such as IGF-1, PTHrP, and Indian hedgehog (Ihh), is also not understood, and requires a systematic investigation. Other challenging questions include placental and mammary calcium transfer under diabetic conditions, long-term impact of GDM in both mothers and neonates, and interplay between classical calciotropic hormones and non-classical mineral-regulating hormones, e.g., prolactin, FGF-23, and insulin itself. In addition, because certain antidiabetic agents, including thiazolidinediones and FGF-21, can aggravate osteopenia [99, 100], it is necessary to develop a practice guideline for antidiabetic drug use in patients with diabetic osteopathy.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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