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A new perspective on mechanisms governing skeletal complications in type 1 diabetes

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Abstract

This review focuses on bone mechanobiology in type 1 diabetes (T1D), an area of research on diabetes-associated skeletal complications that is still in its infancy. We first provide a brief overview of the deleterious effects of diabetes on the skeleton and of the knowledge gained from studies with rodent models of T1D. Second, we discuss two specific hallmarks of T1D, low insulin and high glucose, and address the extent to which they affect skeletal health. Third, we highlight the mechanosensitive nature of bone tissue and the importance of mechanical loading for bone health. We also summarize recent advances in bone mechanobiology that implicate osteocytes as the mechanosensors and major regulatory cells in the bone. Finally, we discuss recent evidence indicating that the diabetic bone is “deaf” to mechanical loading and that osteocytes are central players in mechanisms that lead to bone loss in T1D.

Keywords

mechanical loading; bone; osteocytes; high glucose; insulin

Introduction

Type 1 diabetes mellitus (T1D), also known as juvenile diabetes for its characteristic early diagnosis in children and adolescents, is a form of diabetes that results from autoimmune destruction of pancreatic insulin-producing β cells. When the numbers of β cells fall below normal levels, the body loses its ability to regulate blood glucose level, thereby developing

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Conflicts of interest

The authors declare no conflicts of interest.

diabetes.¹ Approximately 1.25 million American children and adults, and about 35 million people globally, have this form of diabetes.^{2–5} Moreover, the incidence of T1D is increasing 3–5% annually worldwide, possibly owing to unidentified environmental factors.^{6–10} T1D is also associated with an increase in mortality at every age; by some estimates, mortality rates are five to ten times higher among those with the disease.^{2, 5, 11} The early nature of T1D onset, with average diagnosis age being 14 years, results in a lifetime exposure not only to erratic blood glucose levels but also to increased risk of complications.^{12, 13} The list of diabetes-related complications is extensive and involves nearly every organ system, including the skeleton.^{13–16}

Type 1 diabetes and skeletal complications

Bone, although mechanically rigid in appearance, is not at all a quiescent tissue. It is primarily made up of mineral (hydroxyapatite crystals), collagen (mostly type I collagen, Col 1), and various non-collagenous proteins. While the mineral component makes the bone rigid in order to bear the external mechanical loads exerted during movement, Col 1 and other organic components provide ductility to withstand bending or torsion.^{17, 18} Bone cells within the bone tissue are responsible for the composition of the skeleton and include four types: osteoblasts, which are bone-forming cells; osteocytes, which are matured osteoblasts and the most abundant cells, comprising approximately 90–95% of the permanent bone cell population; osteoclasts, which resorb or break down bone tissue; and lining cells, which cover inactive bone surfaces and play a role in bone mineral homeostasis by maintaining the bone fluids and ion fluxes within the bone porosity.^{19–22} During development, a gain in skeletal mass and changes in skeletal morphology are driven by bone modeling, a process that involves new bone formation. After growth, 5–10% of the adult skeleton is renewed every year by remodeling,²³ a process orchestrated by osteocytes that involves coordinated osteoblast and osteoclast activity, in which small packets of bone is resorbed by osteoclasts followed by the replacement of new healthy bone by differentiated osteoblasts. Remodeling takes place throughout the life span and is essential for calcium (Ca^{2+}) homeostasis and ability of bone to respond and adapt to the mechanical environment. Targeted remodeling can also occur to repair microcracks and local defects that result from wear and tear imposed on bone tissue by mechanical loads.^{24, 25} Impaired bone modeling or remodeling, and the consequent disruption of the balance between these processes, can lead to bone diseases such as osteopenia (low bone density) and osteoporosis (fragile bone with an increased susceptibility to fracture).

The association between diabetes mellitus, hyperglycemia, and osteopenia was first described in the late 1920s by the work of Morrison and Bogan.²⁶ In 1949 Albright and Reifenstein reported the incidence of osteoporosis in patients with long-standing and poorly controlled diabetes, which was followed in 1952 by a case report by Berney reemphasizing the co-occurrence of diabetes and osteoporosis.^{27, 28} Since then, a wealth of clinical and experimental studies have provided evidence that osteopenia is a chronic complication of T1D. Low bone mineral density, specifically in trabecular bone, and reduced bone mass ranging from 5% to more than 21% are observed in diabetic children and adults, which further supports the notion that bone health is impaired because of an inadequate accrual of peak bone mass and bone formation.^{16, 29–43} Childhood and adolescence are critical periods

for skeletal development, and patients affected by T1D at these early ages are thus expected to be especially vulnerable to skeletal changes.⁴⁴ With aging, T1D patients face not only significantly increased risks for osteoporosis and bone lesions, but also a higher incidence of osteoporotic fractures, which places T1D as one of the top 10 risk factors for bone fractures.^{16, 42, 43, 45–47} In addition, over the years, it has become increasingly recognized that fragility fractures and delayed bone healing resulting from low bone strength in T1D are not only clinically relevant, but are also major skeletal complications that significantly reduce the quality of life of T1D patients.^{1, 4, 15, 48–52}

Despite growing clinical and epidemiological evidence that supports the association of T1D with low bone mass and fractures, the mechanisms underlying diabetic osteopenia are still not fully understood. Animal models of T1D have been instrumental in better understanding the etiology of bone loss in T1D. As in diabetic humans, significant bone loss is observed in pharmacological (i.e., alloxan or streptozotocin (STZ) and in genetically predisposed or engineered (i.e., spontaneous diabetic biobreeding (BB) rats, non-obese diabetic (NOD) and Akita mice) rodent models of T1D.^{53–61} Histomorphometric studies and analyses of biochemical markers of bone formation and resorption in T1D animals revealed decreased osteoblast activity that is accompanied by increased, normal, or decreased osteoclast activity.^{53, 54, 56, 58, 62–68} Although reports of the effects of diabetes on bone resorption remains controversial and are likely related to differences in the duration of diabetic status (early versus chronic diabetes), the studies with T1D models consistently suggest that bone loss in T1D is mainly related to impaired bone formation rather than increased bone resorption. It still remains to be further investigated whether osteoclast activation occurs early in the disease progression and contributes to the significant bone loss seen at later time points.

Animal models for diabetes have also been widely used to investigate the effects of T1D on mechanical and material properties of bone and its association with increased bone fragility observed in diabetic patients. These studies have shown that bone structural mechanical properties deteriorate in T1D animals, similar to what has been observed in diabetic patients: weaker bones with lower fracture energy and lower bending and torsional strength.^{56, 69–73} Bone matrix is also altered in T1D animals that display decreased mineral crystal size and perfection and increased osteocalcin levels and glycation.^{73, 74} Glycation or non-enzymatic glycosylation of proteins and lipids is markedly increased in diabetes due to the characteristic high extracellular glucose levels. Glycation typically impairs the native function of the molecules and can be followed by a chain of other chemical reactions that ultimately forms advanced glycation end products (AGEs). Accumulation of AGE-modified proteins and activation of specific cell-surface receptors for soluble AGEs (RAGE) are well known to play key roles in the development of diabetic complications.⁷⁵ Collagen glycation in the bone can increase the stiffness of the collagen network and lead to decline in bone matrix quality, which may be a factor contributing to the observed increase in skeletal fragility and fracture risk in diabetics.^{76–78} Overall, material properties of diabetic bones (independently of bone size and geometry) are inferior with lower yield stress and lower nano-indentation stiffness and hardness compared to healthy bones.^{74, 79} Collectively, these findings from animal studies indicate that T1D, when left untreated, could result not only in lower bone mass, but also mechanically inferior, more fragile, weaker bones.

Low insulin versus high glucose: impact on skeletal health

Low insulin levels and hyperglycemia are hallmarks of T1D and key factors in the pathophysiology of diabetes complications. Given the known anabolic effects of insulin on bone, low insulin levels are logically seen as the main culprit for lower bone mass in T1D.^{59, 80–82} However, the actual role played by insulin in the etiology of skeletal complications in T1D is difficult to distinguish from that of high glucose or other factors that are also associated with T1D, such as altered levels of insulin-like growth factor (IGF), amylin, parathyroid hormone (PTH), and osteocalcin. Insulin replacement therapy has been shown to normalize skeletal turnover and growth in chronic STZ-induced T1D rats and also to accelerate fracture healing in BB rats when applied locally, without affecting glycemic levels.^{53, 83} Yet, studies with adult mice that lack global insulin receptor expression (IRKO-L1) but are made euglycemic by expression of the human insulin receptor transgene in the pancreas, liver, and brain, indicated that reduced insulin receptor signaling in the bone would not be a major factor contributing to reduced bone density in T1D.⁸⁴ Nonetheless, significant changes in bone volume are observed at early postnatal age in osteoblast-specific insulin receptor knockout mice (Ob- IR) and also in IRKO-L1 mice. This finding indicated that alterations in bone mass associated with lack of insulin receptor signaling might depend on the time that it occurs during skeletal development.⁸⁵ Insulin receptor expression is low or absent in osteocytes and the beneficial effects of insulin therapy on the skeletal system in T1D are most likely attributed to its action on osteoblasts.⁸⁵ Insulin promotes osteoblast proliferation and differentiation, and is required for normal postnatal bone acquisition.^{85–90} Insulin signaling in osteoblasts has also been shown to play an essential role in regulating whole-body glucose homeostasis by increasing levels of the uncarboxylated form of osteocalcin, which releases osteocalcin from the bone and functions as a hormone that promotes insulin production and secretion by the pancreas, and increases insulin sensitivity and energy expenditure.^{86, 90–92} Therefore, rescuing insulin signaling in osteoblasts would not only improve osteoblast function and promote bone formation in T1D, but would also assist in regulating glucose homeostasis.

In addition to its recognized effects on bone formation, insulin also affects bone resorption.⁹¹ A direct effect of insulin on osteoclasts is indicated by insulin receptor expression in these cells and by findings showing that insulin treatment inhibits *in vitro* osteoclast resorption activity in a dose-dependent manner.⁹³ Insulin also indirectly affects osteoclast function when it signals through osteoblasts, which, in contrast, favor bone resorption.⁸⁶ Insulin signaling in osteoblasts decreases expression of osteoprotegerin (OPG; a negative regulator of osteoclast function) and promotes the ability of osteoclasts to acidify the bone extracellular matrix.⁸⁶ The low pH generated by osteoclast activity is not only required for proper bone resorption, but is also sufficient to decarboxylate osteocalcin.⁸⁶ In this regard, while insulin uses the osteoblast as a direct target to regulate whole-body glucose homeostasis, it is the indirect insulin action on the osteoclast to promote bone resorption that represents the actual mechanism that regulates osteocalcin activation.^{86, 91}

Normalization of glycemia is the most prominent downstream effect of insulin treatment in T1D. Insulin-independent effects of high glucose on bone function have to be considered in lieu of factors that lead to skeletal complications in diabetes. Emerging evidence

demonstrates that the effects of T1D on osteoblast differentiation and function can be replicated *in vitro* by exposure to high glucose.^{63, 66, 81, 94–98} *In vitro* findings, however, are somewhat contradictory and discrepancies have been attributed to differences in experimental design, such as in (1) concentration and duration of the exposure to high glucose, (2) cell seeding density, and (3) culture conditions, with or without ascorbic acid and β -glycerophosphate. A few studies have shown that *in vitro* exposure to high glucose increases osteoblast proliferation, alkaline phosphatase activity, and collagen I mRNA levels, and decreases osteocalcin mRNA levels; other studies demonstrated that high glucose exposure causes reduced alkaline phosphatase activity in primary rat osteoblasts, increases mineralization, mRNA levels of osteocalcin, Runx2 (Runt-related transcription factor 2), bone sialoprotein, and RANKL (receptor activator of nuclear factor κ -B ligand), and also decreases OPG expression in human osteoblasts.^{94–97, 99} Osteoclast differentiation and function seem to also be affected by high extracellular glucose,^{100–103} exposure to which has been shown to suppress osteoclast-related genes, such as *RANK* and *Ctsk* (cathepsin K), and inhibit TRAP (tartrate-resistant acid phosphatase) activity, RANKL-induced osteoclast formation, caspase-3 activity, reactive oxygen species (ROS) production, and osteoclast migration.^{100, 102, 103} Prolonged exposure to high glucose can also indirectly alter the function of osteoblasts and osteoclasts by enhancing AGE generation and glycation of the bone matrix. For example, integrin-mediated adhesion of osteoblasts to collagen matrix as well as osteoblast differentiation are significantly inhibited by culturing the cells in an AGE-modified type 1 collagen substrate.^{104, 105} Combined exposure to high glucose and AGE has been shown to inhibit osteoblast mineralization *in vitro*, through a glucose-mediated increase in expression of the AGE receptor (RAGE).¹⁰⁶ Osteoclast activity and differentiation have also been shown to be impaired in AGE-modified bone collagen matrix.¹⁰⁷

So far, little attention has been paid to how T1D is affecting osteocytes. Given that osteocytes are the master orchestrators of bone remodeling (see the above sections “Bone health: mechanical loading is the key” and “Bone mechanobiology”), it is inconceivable to ignore the potential contribution of these cells to skeletal complications associated with T1D.^{19–21} Although *in vitro* studies investigating the effects of insulin on osteocytes are scarce, there is some emerging evidence demonstrating the effects of high glucose and also of AGEs on osteocytes. Tanaka and colleagues demonstrated that exposure to high glucose increases sclerostin expression in osteocytes, whereas exposure to AGEs not only increases sclerostin but also decreases RANKL expression.¹⁰⁸ Given the known anti-anabolic effects of sclerostin on bone formation and the role of RANKL in regulation of bone resorption, these findings indicate a direct participation of osteocytes in mechanisms that can lead to diabetic osteopenia. Moreover, recent studies performed by our laboratory and others have shown that exposure to high glucose impairs one of the essential functions of the osteocyte—to properly respond to mechanical loading, which is required for appropriate bone homeostasis.^{98, 109} These studies showed that a high-glucose environment alters (1) expression of key molecular mediators of osteocyte mechanosignaling and transduction (pannexin 1 channels and purinergic receptors); (2) release of mechanosignaling molecules (adenosine triphosphate, ATP; prostaglandin E₂, PGE₂); (3) Ca²⁺ signaling in response to mechanical stimulation; and (4) regulation of apoptosis in response to mechanical stimulation.^{98, 109–111} These findings shine a spotlight on osteocytes and open a new avenue

for investigation where the impact of diabetes on bone mechanobiology would take center stage in mechanisms that lead to skeletal complications in T1D.

Bone health: mechanical loading is the key

Since the studies by Julius Wolff in 1982, bone has been recognized as a distinctly mechanosensitive tissue.¹¹² It is a fluid-filled organ that is continuously responding to repetitive mechanical loading imposed by daily physical activities, with modeling (formation or reshaping) and remodeling (removal and replacement).^{112–114} While long-term removal of mechanical loading because of immobilization, bed rest, or space flight results in increased bone remodeling and bone loss accompanied by reduced matrix protein production and elevated osteocyte apoptosis, an increase in repetitive mechanical loading, such as in exercise and experimental anabolic loading, leads to increased bone formation and decreased bone remodeling.^{115–123} Thus, the ability of osteocytes to perceive and respond to mechanical stimulation is essential to orchestrate bone formation by osteoblasts and bone resorption by osteoclasts. Among the many proposed mechanical signals imposed on bone cells, the ones that have received the most attention are the drag forces generated by load-induced interstitial fluid flow (oscillatory flow) and solute movement through the pericellular matrix that create tensile forces along the transverse elements supporting the pericellular matrix, and the cellular strain amplification at focal attachment sites along the cell process, as Piekarski and Munro recognized almost four decades ago.^{124–130}

Bone mechanobiology

How bone recognizes and responds to mechanical loading is a century-old mystery. Mechanobiology of bone consists of the following components: (1) cellular mechanosensing and activation, and (2) signaling and transduction. This is the cascade of events by which biophysical forces are converted to biochemical signals and cellular responses.¹¹³

Mechanosensing and activation

It is widely accepted that mechanosensors or receptors are located either on the apical or basal surface of the cell (transmembrane proteins on the cell surface (pericellular matrix), stress-activated channels, mechanosensitive ion channels, integrins, and focal adhesions) and that the signal transmission is carried out via the cell cytoskeleton. The key question in the field relates to which cell types in the bone are actually the main mechanosensing cells. Until recently, osteocytes were viewed as passive cells.^{19–21} However, a wealth of recent data from ultrastructural studies of the bone lacunar canalicular system by Cowin, Schaffler, Weinbaum, and colleagues have advanced the knowledge needed to not only identify osteocytes as the key mechanosensing cells in the bone, but also to help answer the fundamental question of how they sense the signals from the mechanical environment.^{19, 21, 130–132} Recent studies demonstrated that the osteocyte cell processes are much more rigid than the cell body, owing to their tightly cross-linked actin bundles, making them more mechanosensitive compared to the cell body in response to fluid shear stress.^{21, 133, 134} This concept was supported by multiple reports demonstrating that the unique local structural arrangement and strain amplification along the cell processes— $\alpha_v\beta_3$ integrin attachment sites and transverse tethering elements (proteoglycans within the

canaliculi, namely CD44 and perlecan)—allow the osteocyte to respond to mechanical stimulation in a polarized manner to activate mechanotransduction and signaling pathways.^{127, 133, 135–137}

Mechanosignaling and transduction

The cell cytoskeleton is well recognized as a signal transmission structure. It is commonly observed that bone cells respond to fluid shear stress by increased formation of actin stress fibers, releasing signaling molecules that are essential for bone homeostasis (ATP, PGE₂, and vascular endothelial growth factor (VEGF), transiently increasing intracellular Ca²⁺ levels, altering expression of gap junctions, and modifying both cytoskeletal and focal adhesion proteins.^{138–145} When the cytoskeleton is compromised (cytochalasin B and D treatment, chelating intracellular Ca²⁺, or blocking α -actinin), cells fail to realign, produce early response gene *c-fos*, express COX-2 (a key enzyme in producing prostaglandin), release PGE₂, and form flow-induced stress fibers.^{139–141} Compromising focal adhesions (specifically blocking integrin-fibronectin binding with Arg–GLy–Asp–Ser (RGDS) peptides) results in decreased flow-induced COX-2 induction and PGE₂ release.¹⁴⁶ Finally, compromising the pericellular matrix (enzymatic treatment to remove cell surface-associated proteoglycans) eliminates flow-induced PGE₂ release, while Ca²⁺ signaling is unaffected.¹⁴² These data highlight the exquisite interplay between mechanosensing, signaling, and transduction.

Mechanosignaling molecules: release mechanisms and pathways

In osteoblasts and osteocytes the flow-induced synthesis and release of PGE₂, one of the key extracellular mediators in bone biology, rely on the integrity of the pericellular matrix, cytoskeleton, and focal adhesions.^{140, 142, 146} On a similar note, extracellular ATP and its cell-surface receptors (purinergic receptors, P2Rs) are regarded as key osteoblast and osteocyte mechanosignaling molecules and mechanotransducers, respectively.^{133, 147} P2Rs are known modulators of osteoblast function.^{148, 149} In addition, activation of P2Rs by ATP released from flow-stimulated osteocytes and osteoblasts has been implicated in flow-induced PGE₂ release.^{144, 150} However, the mechanisms and pathways involved in ATP release from bone cells remain to be clearly defined. Studies with osteoblasts indicate that flow-induced ATP release occurs through a vesicular mechanism dependent on Ca²⁺ influx through opening of mechanosensitive cation-selective channels (MSCCs), whereas studies with osteocytes suggest connexin 43 (Cx43) hemichannel as an efflux pathway for flow-induced ATP release (Fig. 1A).^{144, 150} Furthermore, flow-induced opening of Cx43 hemichannels has also been implicated as a pathway for PGE₂ release from osteocytes (Fig. 1A).^{151, 152} However, recent data implicate the pannexin 1 (Pannx1) channel and P2X7R (P2R subtype), rather than the Cx43 hemichannel in flow-induced ATP and PGE₂ release from bone cells (Fig. 1B).^{98, 153, 154} Pannx1 channels are quite unique in their properties in that (1) they form large cell-surface channels that provide a conduit for exchange of ions and molecules (MW up to 1 kDa); (2) they are mechanosensitive, and (3) they can be indirectly activated by ATP via stimulation of P2X7R.^{155–159} In other cell types, P2X7R has also been shown to mediate ATP release, which involves opening of Pannx1 channels.^{156, 160, 161} Since both osteoblasts and osteocytes co-express P2X7R and Pannx1, the existence of such a

functional P2X₇R–Pann1 interplay provides a mechanism for controlled regenerative ATP-induced ATP release and amplification of intercellular signaling in response to mechanical loading (Fig. 1B).^{98, 153, 154, 162–164}

Are diabetic bones “deaf” to mechanical loading?

The current view is that osteopenia in T1D is mainly caused by a reduction in osteoblast numbers, function, and/or maturation.^{40, 68, 165, 166} However, recent advances in bone biology and numerous reports highlighting the essential roles of osteocytes in bone homeostasis emphasize the importance of these cells not only as targets, but also as major players in bone pathophysiology.^{19, 21, 167} Providing that mechanical loading is required for maintenance of bone health (see the above sections “Bone health: mechanical loading is the key” and “Bone mechanobiology”), we advanced the idea that altered osteocyte mechanosignaling and transduction are at the center of mechanisms that lead to bone loss in T1D.^{109, 110} Our studies demonstrated that the P2X₇R–Pann1 mechanosignaling complex is targeted by diabetes; significant downregulation of Pann1 and P2X₇R was observed in long bones of young adult T1D Akita mice and also in osteocytes exposed to high glucose *in vitro*.^{98, 109, 110} Moreover, flow-induced ATP release and Ca²⁺ signaling in osteocytes are markedly reduced in a high-glucose environment, which further supports the notion that mechanically induced ATP signaling in osteocytes is impaired in diabetic bone (Fig. 2).^{98, 109, 110} Concurrently, studies by Parajuli and colleagues showed that exposure to high glucose reduced flow-induced PGE₂ release (Fig. 2) and soluble RANKL (sRANKL) secretion from osteocytes, and altered regulation of osteocyte apoptosis in response to mechanical stimulation.¹¹¹ Considerable reduction of load-induced anabolic response in ulnae of adult Akita mice was also observed.¹¹¹ Overall, these *in vitro* and *in vivo* findings indicate that diabetic bones respond differently to mechanical load compared to healthy bones. Given that a proper response to mechanical loading is essential for maintenance of bone health, “deafness” to mechanical loading (Fig. 2) may be at the center of mechanisms that lead to skeletal dysfunction in T1D (Fig. 3).

Clinical relevance

Current management of skeletal complications in patients with diabetes relies mainly on use of pharmacological and nonpharmacological osteoanabolic approaches that are also being used to treat bone diseases in nondiabetic patients.^{15, 168, 169} Previous translational studies stemming from *in vitro* models to *in vivo* diabetic models have made headway in developing these therapeutic approaches that specifically target osteoblasts and/or osteoclasts. Among those are the treatments with insulin, PTH, oxytocin, zinc supplement, and sclerostin antibody that have stimulatory effects on osteoblastogenesis and modulatory effects on osteoclast function and formation, which improve bone loss and/or healing.^{14, 170–173} With the emergence of the osteocyte as a key player in diabetic skeletal complications, we expect that future preclinical and translational studies will focus on development and testing of novel osteocyte-targeted therapeutic interventions, either as an alternative or in combination with osteoanabolic approaches to improve treatment of skeletal dysfunction in diabetic patients.

Conclusion

Previous studies have yielded a wealth of information about mechanisms underlying the adverse effects of T1D on bone homeostasis. Current findings have brought a new level of understanding and advanced several promising leads in unraveling the mystery behind skeletal dysfunction associated with T1D. However, there is still much to be learned. In this review we advanced the view on the osteocyte as a central player in mechanisms contributing to diabetic osteopenia. Osteocytes are major orchestrators of bone remodeling and their function is tightly regulated by mechanical loading that arises from daily physical activities. Better understanding of how the diabetic environment affects the function of osteocytes and their interactions with osteoblasts and osteoclasts are essential to gain an integrated perspective of mechanisms that lead to skeletal complications in T1D.

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Figure 1.

Schematic diagrams depicting the two main pathways that have been proposed to mediate fluid shear stress–induced ATP and PGE₂ release in osteocytes. (A) Fluid shear stress (1) activates the osteocyte and, through a mechanism involving protein kinase C (PKC) (2) or PI3K signaling and activation of α5β1 integrin (3), leads to opening of Cx43 hemichannels and ATP release, which is then followed by PGE₂ release (4). (B) Fluid shear stress (1) activates mechanosensitive Panx1 channels on osteocyte processes, leading to ATP efflux (2). Released ATP diffuses, binds, and activates P2X7Rs (3), which results in Ca²⁺ influx and interaction of P2X7R with Panx1 (4) that opens the Panx1 channel (5) and induces ATP efflux. Autocrine P2X7R activation and formation of the functional P2X7R–Panx1 complex generate a feedforward mechanism of ATP-induced ATP release from stimulated cells (6) that amplifies paracrine signaling (7) within the osteocyte network.

Figure 2.

Schematic representation of mechanosignaling events mediated by P2X7R–Panx1 in (A) healthy and (B) diabetic bone. (A) In healthy bone, fluid shear stress (FSS) (1) is transmitted through the pericellular matrix (PCM) and triggers an increase in intracellular Ca^{2+} levels ($[\text{Ca}^{2+}]_i$) (2) and ATP efflux through mechanosensitive Panx1 channels (3). Released ATP activates P2X7R (4), which further increases $[\text{Ca}^{2+}]_i$ (5) and opens Panx1 channels, resulting in additional ATP release followed by PGE_2 release (7); P2X7R–Panx1-mediated ATP-induced ATP release amplifies FSS-induced osteocyte stimulation (6 and 7). (B) In diabetic bone, prolonged exposure to high extracellular glucose modifies the PCM (i.e., thickening, glycation) and thereby alters FSS transmission (1), which results in lower Ca^{2+} mobilization (2) and ATP release (3). Lower expression and lesser activation of Panx1 and P2X7R result in reduced release of mechanosignaling molecules (ATP/ PGE_2) (5–7), which ultimately translates into lower responses of diabetic bone cells to mechanical loading.

Figure 3.

Diagram illustrating a new perspective on mechanisms involved in T1D-associated skeletal complications. Green arrows: osteocytes in healthy bone respond properly to mechanical loading and coordinate adaptive response of osteoblasts and osteoclasts that are required to achieve skeletal homeostasis. Red dotted arrows: osteocytes in diabetic bone fail to properly respond to mechanical loading and to orchestrate the adaptive response of osteoblasts and osteoclasts, thereby leading to skeletal dysfunction.