



Metformin; an old antidiabetic drug with new potentials in bone disorders

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ABSTRACT

The prevalence of diabetes mellitus especially type 2 diabetes mellitus is increasing all over the world. In addition to cardiomyopathy and nephropathy, diabetics are at higher risk of mortality and morbidity due to greater risk of bone fractures and skeletal abnormalities. Patients with diabetes mellitus have lower bone quality in comparison to their non-diabetic counterparts mainly because of hyperglycemia, toxic effects of advanced glycosylation end-products (AGEs) on bone tissue, and impaired bone microvascular system. AGEs may also contribute to the development of osteoarthritis further to osteoporosis. Therefore, glycemic control in diabetic patients is vital for bone health. Metformin, a widely used antidiabetic drug, has been shown to improve bone quality and decrease the risk of fractures in patients with diabetes in addition to glycemic control and improving insulin sensitivity. AMP activated protein kinase (AMPK), the key molecule in metformin antidiabetic mechanism of action, is also effective in signaling pathways involved in bone physiology. This review, discusses the molecules linking diabetes and bone turnover, role of AMPK in bone metabolism, and the effect of metformin as an activator of AMPK on bone disorders and malignancies.

1. Introduction

World health organization has reported that about 422 million people all over the world suffer from diabetes [1]. Diabetes mellitus (DM) is a metabolic disorder characterized by high blood glucose that affects many organs in the body. Ability of insulin production by pancreas in type 1 diabetes mellitus (T1DM) and usage of insulin and its receptors in type 2 diabetes mellitus (T2DM) are demolished [2,3]. Although, patients with T1DM are at higher risk of bone fractures due to low bone mineral density (BMD), there is an increased incidence of fractures in T2DM patients despite high body mass index (BMI) and normal or even high BMD [4]. Several factors may lead to this fact including renal failure, antidiabetic drugs, and increased prevalence of falls [5]. Previous studies have investigated the biochemical bone turnover markers in T2DM patients and have reported lower bone formation markers in these patients [6]. Furthermore, in vitro studies have revealed that hyperglycemic conditions leads to adipogenic differentiation rather than osteogenesis as well as impaired growth and enhanced apoptosis in osteoblasts [7]. T2DM is characterized by hyperglycemia, hyperinsulinemia, and insulin resistance [8], but the exact mechanism illustrating their effects on bone metabolism is not clear. However, it seems logical that antidiabetic medications and glycemic control may have beneficial effects in bone tissue. Antidiabetic

medications mainly include insulin therapy in T1DM and hyperglycemic correction and insulin sensitization in T2DM by oral antidiabetic drugs (metformin, thiazolidinediones, and sulfonylureas) [9]. Insulin therapy in T1DM patients have been shown to increase bone mass possibly by direct anabolic effects of insulin on bone metabolism [3,10], although, there are some reports about the association of insulin treatment with risk of bone fractures [11]. Thiazolidinediones (TZDs) are a class of antidiabetic agents such as rosiglitazone and troglitazone that exert their effects by activating peroxisome proliferator-activated receptor γ (PPAR γ). Despite their beneficial effects in insulin sensitizing and diabetes glycemic control, clinical and epidemiological studies have shown increased fracture risk in patients receiving TZDs, particularly in women [12,13]. The exact mechanism for this adverse effect on bone is not clear, but one reason is the negative effect of PPAR γ activation on bone remodeling. Activation of PPAR γ by TZDs stimulates the differentiation of precursor mesenchymal stem cells (MSCs) into adipocyte lineage, rather than osteoblast formation. Therefore, TZDs increase the risk of fractures while they enhance insulin sensitivity [14,15]. On the other hand, metformin an antidiabetic drug belonging to bigunide compounds have shown to shift the progenitor cells into osteoblasts [16]. The antidiabetic effects of metformin occurs by stimulation of AMP activated protein kinase (AMPK) as a result of blocking the mitochondrial respiratory chain and enhanced

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AMP/ATP ratio [9]. Metformin has direct osteogenic effects on bone through AMPK and Runt related transcription factor 2 (Runx2) and indirect effects by hyperglycemic correction [16]. In this study, we will review and discuss the molecules linking diabetes and bone metabolism, molecular mechanisms of metformin in bone formation, and preclinical and clinical application of metformin in various bone disorders and malignancies.

2. Diabetes and bone

The worldwide prevalence of DM especially T2DM is increasing and there are growing evidences that DM can be the underlying cause and risk factor of osteoporotic fractures [1,17]. Diabetes affects bones through glucose metabolism impairment, disruption of bone microvascular function, glucose oxidative derivatives and muscle endocrine function [10]. Bones are heavily related to glucose metabolism and obviously osteoblast proliferation needs glucose [18]. In general, diabetic patients have lower bone quality in comparison with non-diabetic individuals and it has been reported that DM can affect bone tissue (osteopenia and osteoporosis) due to hyperinsulinemia, reduced serum levels of IGF-1, presentation of advanced glycosylation of end-products (AGEs) especially in collagen following by hyperglycemia, decreased levels of osteocalcin, renal failure, hypercalciuria, microangiopathy, and inflammation [19]. It has been suggested by some in vitro studies that AGEs have inhibitory effects on osteoblastic proliferation [20] and stimulatory effects on osteoclastic resorption [21]. Pentosidine as a member of AGEs has been isolated from bone in diabetic rats. In fact, AGEs accumulation which impairs osteoblastic function [22,23], can increase risk of fracture and postpone fracture healing in type 2 diabetes due to collagen defect induction and decreased collagen amounts [24–26]. Furthermore hyperglycemia and its following consequences have negative effects on osteocalcin production which is responsible for matrix maturation and bone mineralization. Osteocalcin secretion in insulin-activated osteoblasts has stimulatory effects on proliferation of β -cells in pancreas, insulin sensitivity, insulin secretion, energy outlay [27,28], and also testosterone production which of its kind is an osteogenic factor [29]. Osteoblasts and adipocytes have common ancestor pluripotent cells called bone marrow progenitor cells (BMPC) of mesenchymal origin. BMPCs are differentiated into osteoblasts by expression of transcription factor Runx2 [30] and on the contrary by peroxisome proliferator activated receptor- γ (PPAR γ), BMPCs are differentiated into adipocytes [31]. PPAR γ 2 can play an important role in pathophysiology of osteoporosis in diabetic patients [32]. Besides, hyperglycemia derives mesenchymal stem cells (MSCs) toward adipogenesis and declines osteoblastogenesis in vitro. In addition, the induction of reactive oxygen species followed by hyperglycemia has an important role in diabetic complications which affects not only osteoblastogenesis, but also stimulates osteoblastic apoptosis [33]. T1DM results in low bone density, as duration of diabetes has an important role in lowering bone mineral density found among patients with diabetes more than 5 years [34]. Furthermore, in T1DM pancreatic β -cells can't produce islet amyloid and preptin which have osteo-anabolic effects in companion with insulin [35]. It has been reported that high bone resorption in poorly controlled T2DM can be normalized by accurate glycemic control [36,37]. Bone tissue quality depends on bone remodeling in order to replace old tissue by osteoclasts with new and more functional bone tissue by osteoblasts [38]. Even high fasting glucose levels can increase the risk of hip fractures [39]. Possible increased risk of falling due to peripheral neuropathy, hyperglycemia and visual impairment caused by diabetes can increase fracture incidence [4,40,41]. Most studies have indicated that in patients with T1DM bone mineral density is decreased [42–45], while, in T2DM patients bone mineral density is normal [46–50] or enhanced [40,51,52]. However, as a fact there is a close correlation between bone fragility and fracture risk with both types of DM regardless of bone mineral density status [32,53]. Patients with T2DM have higher rates of hip [47,54,55], arm

[51,52], foot and ankle fractures [41,56] and the risk of hip fractures in T1DM patients is 7 fold higher than non-diabetics [19]. Insulin signaling and metabolism of glucose have a positive correlation with bone turnover [10]. Several studies have demonstrated that the exact glycemic control can result in enhanced bone turnover (resorption and formation) and poorly glycemic control in diabetic patients can lead to imbalance of osteoblast/osteoclast activity and impaired bone formation [37,57,58]. Furthermore, a study has reported that diabetes is an important risk factor of osteoarthritis [59]. DM may cause serious damages in kidneys, heart, nerves, blood vessels and bone tissue. Routine medication procedures are daily insulin therapy for T1DM patients and common oral insulin sensitizer drugs (metformin, thiazolidinediones, and sulfonylureas) as well as management of life style for T2DM patients [3].

3. Metformin mechanism of action

Metformin as a member of biguanide antidiabetic drugs is a widely used oral medication for treatment of T2DM (non-insulin dependent). After its discovery in 1922, due to glucose lowering effects in trial studies, it was presented into clinical practices in 1950 and it was named Glucophage (glucose eater) in 1957 in France. Although, it has been used over 40 years, its mechanism of action is not fully understood [60]. Metformin has affordable price and it is fairly safe and low risk of hypoglycemia, no incidence of weight gain and few side effects put this drug in first line of oral medication for T2DM [61]. Surprisingly, it has been reported that metformin has no effect on glucose levels in non-diabetic individuals and this represents the idea of using metformin as adjuvant therapy especially in bone disorders [62,63]. Although metformin is first line treatment of T2DM, it can be used in combination with other antidiabetic drugs like sulfonylureas, insulin and DPP-4 inhibitors [64]. Main defined antidiabetic mechanism of action after metformin administration is mitochondrial respiratory chain (complex I) blockage leading to oxidative phosphorylation separation and increased AMP/ATP ratio (Fig. 1). Thereby, increased ratio of AMP/ATP results in 5' adenosine monophosphate-activated protein kinase (AMPK) activation and dozens of other enzymes can be regulated by AMPK [9]. Insulin via activation of insulin receptor (IR) stimulates insulin receptor substrate 1 (IRS1) leading to several reactions resulting in glucose transporter (GLUT) expression in cell membrane and increased glucose uptake [65]. AMPK allosterically activates IR and IRS1 and increases insulin sensitivity [66]. Although, AMPK increases nutrient uptake by boosting insulin sensitivity, it does not act thoroughly like insulin and it inhibits anabolic pathways [67]. Interestingly, AMPK takes body metabolism into catabolic status generating energy and ATP to keep normal cell function and increases insulin sensitivity, glucose and lipid metabolism and declines gluconeogenesis especially in liver. Moreover, through inhibition of lipogenesis and increasing lipid metabolism, metformin reduces hepatic lipids. In general, metformin can influence cell growth, proliferation and apoptosis through several signaling pathways [9]. Metformin is excreted into urine unchanged (not metabolized) and its half-life is about 5 to 6 hours. Elimination of metformin in kidney is by active tubular secretion [68]. Gastrointestinal complications and risk of vitamin B12 and folic acid deficiency especially in long term use of metformin can occur and lactic acidosis which is very rare should be considered in patients with renal impairments [69].

4. AMPK and bone metabolism

AMPK signaling pathway and its stimulatory effects on bone formation and bone mass has an important value in bone physiology [70]. In last decade, AMPK has been distinguished to play an important role in regulation of energy homeostasis in cells and it is an essential mediator for many hormones affecting metabolism of protein, fat and glucose [71–74] (Fig. 1). AMPK activation depends on AMP/ATP ratio

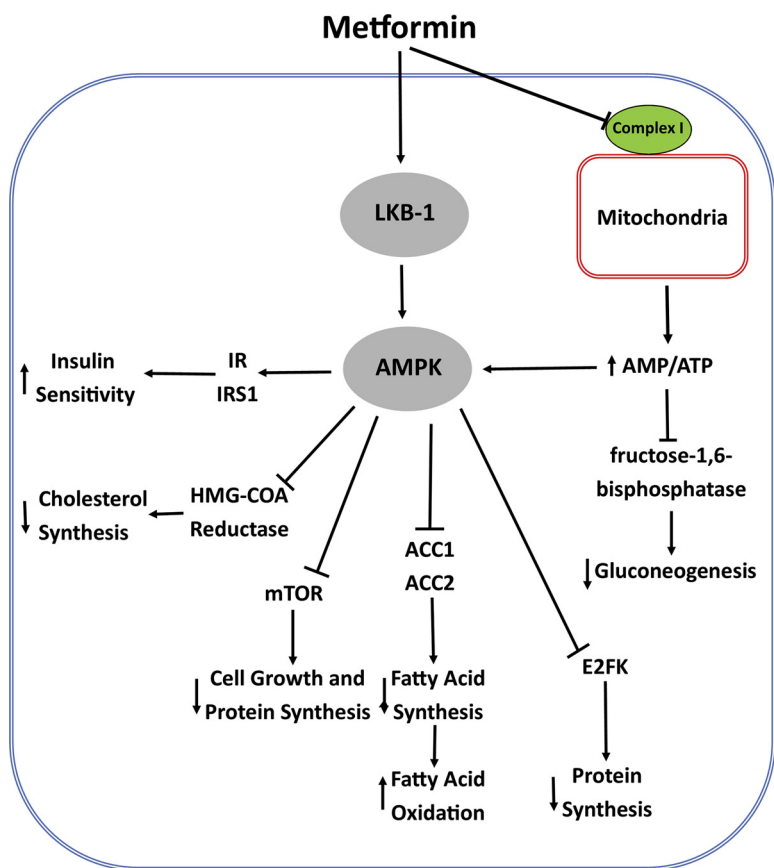


Fig. 1. The multiple pathways in which metformin affects cellular metabolism. Metformin inhibits complex I of mitochondrial respiratory chain, leading to increased AMP/ATP levels and as a result activation of AMPK. Metformin can also activate AMPK through direct activation of LKB1. AMPK allosterically activates IR and IRS1 and increases insulin sensitivity. AMPK switches off ATP consuming pathways such as protein, cholesterol and fatty acid synthesis and switches on ATP production pathways such as glucose uptake and fatty acid oxidation. Enhanced AMP/ATP ratio also inhibits fructose-1,6-bisphosphatase and as a result suppression of gluconeogenesis. ACC1, Acetyl-CoA carboxylase 1; ACC2, Acetyl-CoA carboxylase 2; E2FK, Elongation 2 Factor kinase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IR, Insulin Receptor; IRS1, Insulin Receptor Substrate 1; LKB1, tumor suppressor kinase; mTOR, mammalian target of rapamycin.

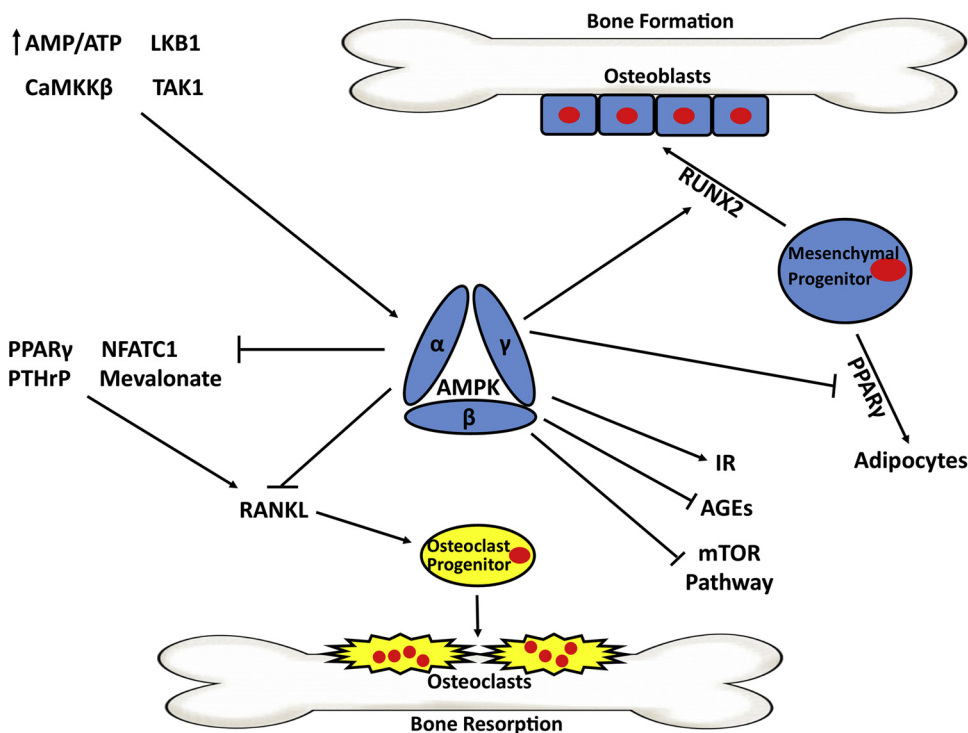


Fig. 2. AMPK and its regulatory effect on bone homeostasis. AMPK activation is under influence of AMP:ATP ratio, tumor suppressor kinase LKB1, CaMKKβ and TAK1 pathways. AMPK affects bone formation through osteoblast-specific transcription factor Runx-2 activation, AGEs down-regulation and IR allosteric activation leading to osteoblastogenesis. PPARγ2 suppression by AMPK has inhibitory effects on adipogenesis. PPARγ1, NFATc1, PTHrP and mevalonate down-regulation by AMPK signaling pathway which results in RANKL suppression can inhibit osteoclastogenesis. AMPK also suppresses mTOR signaling which is hyper activated pathway in tumor cells. AGEs, advanced glycosylation end-products; CaMKKβ, Calmodulin Kinase Kinase β; IR, Insulin Receptor; LKB1, Tumor suppressor kinase; mTOR, Mammalian target of rapamycin; NFATc1, Nuclear factor of activated T-cells, cytoplasmic 1; PPARγ, Peroxisome Proliferator-activated Receptor γ; PTHrP, Parathyroid Hormone-related Protein; RANKL, Receptor activator of nuclear factor kappa-B ligand; Runx-2, Runt-related transcription factor 2.

and upon activation; it turns off anabolic pathways and switches on catabolic pathways within the cell [75–77]. In fact there is two probable mechanisms for activation of AMPK: a) enhanced AMP/ATP ratio and b) activation by signaling and regulatory subunit of AMPK [9]. In the process of signaling activation there are three kinase/phosphatase

enzymes (LKB1, CaMKKb and TAK1) that may have regulatory effects on AMPK [78–80]. AMPK has three subunits (α, β, γ) that can be activated by AMP adherence to α subunit [81]. In general, α, β and γ subunits of AMPK play an important role in the type and speed of AMPK regulation [82,83]. There are α1, α2, β1, β2, γ1, γ2 and γ3 subunits

encoded by 7 genes forming 12 possible AMPK heterotrimers in different regulations [84]. Catalytic process is regulated by α subunit. β subunit not only has glycogen binding domain, but also it can bridle α and γ subunits by tethering domain, and γ subunit is responsible for regulatory nucleotides binding [80,85]. Energy production and catabolic pathways promotion by AMPK generates energy and ATP to keep normal cell function and also it can up-regulate transcription of glucose transporter 4 (GLUT4) genes [86,87]. In fact, as an important phenomenon, AMPK restricts energy consumption to ensure cell survival. In other words, AMPK rations energy in cell and for this purpose, it inhibits protein synthesis and cell growth and also it adjusts cell cycle arrest through down-regulation of mammalian target of rapamycin (mTOR), which is hyper-activated in most tumor cells [88–91]. The regulation of metabolic pathways by AMPK occurs by phosphorylating metabolic enzymes involved in glucose, glycogen and lipid metabolisms [92–98]. A study has reported that AMPK can stimulate osteogenesis in MC3T3-E1 cells and suppress adipogenesis in 3T3-L1 cells via AMPK-Gfi1-OPN axis pathway [99]. Adipocytes and osteoblasts have common cell progenitors known as mesenchymal stromal cells (MSC) or bone marrow stromal cells (BMSC). Regulation of Runx2 and a newly discovered pathway, Wnt/b-catenin, by AMPK can differentiate MSCs into osteoblasts (osteoblastogenesis), while, expression of PPAR γ -2 suppresses osteoblast differentiation and make MSCs differentiate into adipocytes [70,100,101] (Fig. 2). PPAR γ -1 isoform can establish osteoclastogenesis and bone resorption by increasing receptor activator of nuclear factor kappa-B ligand (RANKL) and c-FOS signaling [15,102]. Interestingly, AMPK decreases adipogenesis in vitro by b-catenin phosphorylation and PPAR γ suppression [103]. Furthermore, the activity of PPAR γ can be modified directly by AMPK via phosphorylation [104]. Activation of AMPK reduces bone resorption via suppression of the nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) [105,106]. Another study has suggested that AMPK can suppress RANKL-induced osteoclast formation [107]. It has been shown that osteoclasts are derived from multinucleated giant cells (monocyte-macrophage lineages) in the presence of two cytokines, M-CSF and RANKL [108]. Mevalonate pathway which plays a role in pernylation of regulatory proteins like Ras and Rho GTPase, has a negative influence on bone tissue and it has been reported that AMPK can adjust mevalonate pathway through suppression of HMG-COA reductase [109,110].

5. Metformin and bone disorders

Bone diseases may not get as much attention as other major diseases like cardiovascular diseases and cancers, but they are common and costly and they can become a chronic burden in both individuals and society. As a clear example, estimated occurrence of only osteoporotic fractures in Europe was 2.7 million in 1990 and estimated direct cost in 2004 was €36 billion [111]. Other regions of the world are also involved because of the increasing number of the elderly. Annual osteoporotic fractures in the USA is predicted to cost around \$25.3 billion by the year 2025 [112].

Bone as a highly dynamic tissue is resorbed and rebuilt mainly by 3 cell types including osteoblasts, osteoclasts, and osteocytes, which has a delicate balance between resorption and rebuilding process in normal skeleton. Osteoblasts and osteoclasts, differentiated from mesenchymal and hematopoietic progenitor cells, are responsible for new bone creation and old bone removal. Bone marrow stromal cells (BMSCs) can differentiate into three types of cells including chondrocytes, osteoblasts and adipocytes [113–115]. The hematopoietic progenitor cells of monocyte-macrophage can differentiate into osteoclasts, multinucleated giant cells, which are responsible for bone resorption [108]. Osteocytes are the major parts of bone matrix and it has been found they can contribute in bone remodeling through regulation of osteoblasts and osteoclasts. The balance between bone resorption and bone formation is so important for skeletal hemostasis [3]. Bone tissue quality depends on bone remodeling in order to replace old tissue by

osteoclasts with new and more functional bone tissue by osteoblasts [38]. By aging and some bone diseases, the balance between bone resorption and bone formation will be disoriented and as a result, fracture may happen. Treatment for bone disorders requires the exact understanding of the biology and physiology of osteoclasts and osteoblasts. Drugs that inhibit osteoclasts can be used for treatment of osteoporosis, Paget's disease and bone inflammation associated with rheumatoid arthritis or periodontal diseases. Resorption by osteoclasts takes about 3 weeks per site and on the contrary bone formation by osteoblasts takes about 3 to 4 months. In some people after age 40, the balance begins to shift toward bone destruction and bone destruction outpaces bone formation leading to bone loss called osteoporosis [116].

Bones can be affected by many diseases like diabetes, chronic liver diseases, chronic kidney diseases, malnutrition, gastrointestinal disorders, metastatic cancers and metabolic disorders [117–119]. As discussed above diabetic patients are at higher risk of bone fractures and thus it seems that antidiabetic medications may have beneficial effects in bone disorders. Standard treatment for DM includes insulin therapy, biguanides (metformin), thiazolidinediones (pioglitazone, rosiglitazone, troglitazone), sulfonylureas (glibanclimide, glimepiride) and DPP-4 inhibitors (sitagliptin, vildagliptin).

Recent studies have shown that metformin can be osteogenic in vitro following by activation of AMPK resulting in osteoblastic cells differentiation, bone matrix synthesis, and also osteoblasts proliferation [120–124]. Metformin oral administration has osteogenic effects both in vivo and in vitro and it improves bone healing in non-diabetic animals by increasing osteoblast specific transcription factor (Runx2) and AMPK activation possibly results in osteoblastic differentiation of bone marrow progenitor cells [16]. Osteoblastic differentiation after metformin treatment through activation of Runx2 may occur via AMPK/upstream stimulatory factor-1/small heterodimer partner signaling cascade. Metformin has other effects on osteoblasts by preventing adipogenic differentiation factor, PPAR γ [125]. Both in vitro and in vivo studies have demonstrated that metformin increases type I collagen synthesis and osteocalcin expression. Moreover, it has been shown that metformin stimulates the regeneration process in bone lesions both in diabetic and non-diabetic rats [16]. Deleterious effects of high glucose and AGEs on osteoblasts can also be blocked by metformin. Decreased osteoblastic cellular proliferation, ALP activity, calcium deposition and increased ROS production along with apoptosis caused by high glucose levels would be reversed by metformin. The expression levels of Runx2 and IGF-1 were also up-regulated by metformin. Same results were observed when osteoblastic cells were treated with metformin after AGE exposure. The expression of AGE receptors (RAGE) was declined after metformin treatment that may be the possible mechanism for metformin anti-AGE action [123,126,127]. In addition, AGEs can be decreased by catabolic pathways stimulation followed by AMPK activation [75,76]. Metformin also ameliorated the antiproliferative effects of high glucose in osteoblastic cells via suppression of osteocalcin and osteoprotegerin [128]. Tolosa and colleagues in another study reported that metformin treatment in insulin-deficient diabetic rats can increase Runx2/PPAR γ ratio and decrease RAGE expression resulting in reversal diabetic negative effects on osteogenesis and bone alteration [129]. It has been showed that increased proliferation and differentiation of osteoblast-like cells (UMR106 and MC3T3E1) is accompanied with elevated type-I collagen production and ALP activity. Furthermore, the osteogenic effects of metformin in these cells was presumably mediated by activation of extracellular signal-regulated kinase (ERK) and induction of inducible nitric oxide synthases (e/iNOS) [121]. In osteoclasts, metformin can suppress RANKL signaling and it can increase osteoprotegerin expression by osteoblasts leading to reduced osteoclasts number and prevention of bone loss [130,131]. In hematopoietic cells, metformin inhibits the development of osteoclasts and prevents macrophages pro-inflammatory responses following by down-regulation of AGE receptor signaling [127,132]. In vivo studies have also reported that metformin has protective effects against bone loss after

ovariectomy in rat. In addition, metformin protects bone mass in oestrogen deficiency. Enhanced bone density and quality caused by metformin in ovariectomized rats probably was mediated by increased expression of osteoblast markers core binding factor $\alpha 1$ and LDL receptor-related protein 5. Moreover, the expression of estrogen receptor α increased after metformin treatment which has positive osteogenic effects [130,133]. It has also been reported that metformin can increase bone density and mineralization in alveolar bones through osteoblast differentiation in ligature-induced periodontitis in rats [134]. Clinical data also confirmed that among diabetic patients, metformin users experience lower risk of fractures in comparison to non-metformin users and those who taking other antidiabetic drugs [135,136].

As discussed above, many clinical and pre-clinical studies have shown the beneficial effects of metformin in osteogenesis, however, there are studies reporting the anti-osteogenic effects of metformin in vivo and in vitro. In a very recent study which was carried out by Qin and colleagues, metformin suppressed ossification and inflammation in fibroblasts harvested from capsular ligament of patients with femoral neck fractures and autoimmune disease, ankylosing spondylitis. The positive effects of metformin on ankylosing spondylitis fibroblasts was mediated by PI3K/Akt and AMPK pathway [137]. Jayabalan and co-workers in another study also showed no osteogenic effects of metformin in ovariectomized C57BL/6 mice [138]. These controversial results may be arise from methodological differences, different concentrations and duration of treatment with metformin, and differences in response to metformin among rodent species.

Many bone diseases can result in bone loss and reduction of bone mass leading to fractures and as we discussed, metformin may have beneficial effects in bone turnover and bone healing. Further studies and investigations in this field might have interesting results. Moreover, according to current studies, most of therapeutic procedures have shown to be insufficient or in some cases ineffective. Therefore, the potential effects of metformin on preserving bones can be considered, especially in treatment of osteopenia and osteoporosis.

6. Metformin and bone cancers

Bone is a hospitable tissue for metastatic cancers especially breast, lung and prostate cancers. There are also primary bone cancers initiating in bone tissue itself, however, invasion of metastatic cancers into bones are more common than primary bone cancers [139]. Any types of bone cells can play role as the exciter or beginner of primary bone cancer. Osteosarcoma is a common primary bone tumor arise from osteoid tissue and it has approximately 10 subtypes [140]. Other primary bone cancers include chondrosarcoma and Ewing sarcoma family of tumors which occur in cartilages (sometimes bone cells get involved) and soft tissues of bone (muscle, fat, fibrous tissue and blood vessel), respectively [139,141]. About 350,000 people in the USA die each year from bone metastasis of breast, prostate and lung cancers and interestingly the bulk of tumorous cells can be found in bones at the death time. In addition, the incidence of metastasis to bone increases if patients with breast or prostate cancers live more than 1 year. Except unbearable pain in occupied bones with tumorous cells, fragility and risk of fractures followed by osteolysis increase in most bone related cancers. Any of primary or metastatic bone cancers can play role in osteolytic process by osteoclast activator signaling and also osteoblastic metastases occurs by tumorous cells production of stimulant factors for osteoblastic proliferation, differentiation and bone formation [142]. Moreover, parathyroid hormon-related peptide (PTHrP) which is the main mediator of osteoclast activation, can be secreted by many cancer cells such as breast, lung, renal and pancreatic carcinoma as well as myeloma, and induces bone destruction and hypercalcemia [143,144]. It has been reported that PTHrP activates osteoclasts by RANKL signaling [145–147]. There is an obvious abnormal coupling bone resorption and formation regardless to types of osteoclastic or osteoblastic involvement in cancers that induce bone disorders [142]. Secondary osteoporosis

following by common primary bone cancers (osteosarcoma, chondrosarcoma, Ewing sarcoma) and bone metastasis of cancers (breast, prostate, lung and kidney), is a major problem [148].

In recent years, studies have demonstrated that metformin aside from its effect on glucose and insulin sensitivity, can also reduce incidence of cancers and tumor growth [149]. Metformin can prevent a wide variety of cancer cells including prostate, breast, pancreas, and colon cancers and in long term use it can decline the risk of tumor induction for diabetic patients [150–152]. AMPK as a key molecule in metformin mechanism of action is involved in other signaling pathways further to metabolic pathways. For example, AMPK inhibits mammalian target of rapamycin (mTOR) pathway, which is hyper-activated in most tumor cells, leading to proliferation and cell growth in cancer cells [43,160,161]. It has also been suggested that up-regulation of AMPK by metformin is expected to be the important mechanism of antineoplastic effects [162]. Some in vitro studies on breast cancer have shown that metformin inhibits proliferation and induces apoptosis via PI3K/Akt/mTOR pathway suppression [163,164]. Our very recent studies also showed that metformin enhances the sensitivity of resistant breast cancer cells to doxorubicin via inhibition of P-gp mediated doxorubicin efflux [153,154].

In bone tissue, activation of AMPK by metformin as a negative regulator of RANKL, has an important role in suppressing osteoclast proliferation and differentiation, thereby, metformin can reduce bone resorption and capability of bone for metastasis [107,155,156]. It has been suggested that metformin suppresses the proliferation of osteosarcoma MG63 cells in vitro probably by AMPK/mTOR/S6 signaling pathway. In addition, metformin can inhibit the migration and invasion of osteosarcoma cells by blocking metal matrix proteinases 2 and 9 (MMP2 and MMP9). Metformin has also destructive effects on osteosarcoma cancer stem-like cells and potentially it can reduce the risk of cancer in continuous use by T2DM patients [157]. It has also been shown that metformin has anticancer effects on osteosarcoma and rhabdomyosarcoma through AMPK activation, resulting in mTOR signaling suppression and it can also chemo-sensitize tumorous cells. But in hypoxic circumstances the efficacy of metformin may be very limited [158]. Another study has indicated that metformin suppresses tumor cell growth through cell cycle arrest and decreased activity of S6K mediated by AMPK. Furthermore, metformin enhances cisplatin effects in treatment of osteosarcoma cells in a p53-independent manner by drug-drug interaction [159]. One of the major obstacles against successful cancer chemotherapy of osteosarcoma cells is the development of multidrug resistance. It has been shown that cancer stem cells are responsible for resistance to chemotherapy. This fact has been confirmed by the resistance to cisplatin in osteosarcoma stem cells. Over-expression of pyruvate kinase M2 (PKM2) is known to be responsible for cisplatin resistance in osteosarcoma stem cells. In a study, Shang and colleagues showed that treatment with metformin enhances the sensitivity of osteosarcoma cancer stem cells to cisplatin by down-regulating PKM2 expression [160]. In another study, Li et al. elucidated the mechanism by which metformin inhibits proliferation and migration of osteosarcoma cells. They indicated that these effects are mediated by suppressing Akt activity as a result of enhanced expression of phosphatase and tensin (PTEN) protein levels [161].

There are several studies reporting that metformin and its stimulatory effects on AMPK can be targeted for breast cancer due to effects of AMPK on reduction of transforming growth factor beta (TGF β) [162]. Moreover, metformin therapy in diabetic type 2 women declines the risk of breast cancer as well in non-diabetic women [163,164]. Epidemiological reports have also shown that metformin reduces the incidence of breast, colon, pancreas and prostatic cancers [152,165,166]. These results suggest that metformin further to its anticancer effects in bone malignancies, have beneficial effects on other types of cancers that potentially can migrate to bone tissue. Above all anticancer reports of metformin, surprisingly it has positive effects on osteoblast proliferation, differentiation and osteogenesis which elucidates the beneficial

effects of metformin on bone turnover and bone healing [16,130,133], suggesting the idea that metformin can be considered as an important therapy for bone destructive disorders even regardless to its anticancer effects.

7. Effect of other antidiabetic drugs on bone

Thiazolidinediones (TZDs) which also known as glitazones are antidiabetic drugs for treatment of T2DM. As PPAR γ selective agonists, TZDs can bind PPAR γ receptors and mimic all the effects of its pathway including insulin sensitizing and adipogenesis by lipid uptake enhancement and free fatty acid reduction [167]. Aside from general side effects like weight gain and cardiovascular problems, this class of drugs has a strong negative effect on bone tissue. Activation of PPAR γ by TZDs make MSCs shift into adipogenesis and adipocyte elevation instead of osteoblast in bone tissue. In fact, TZDs stimulate adipocyte differentiation while they inhibit osteoblast differentiation through PPAR γ [14,168,169]. On the other hand, PPAR γ expression results in osteoclast proliferation and bone resorption and absence of PPAR γ in osteoclasts develops osteopetrosis signs [15]. It has also been found that activation of PPAR γ by TZDs can suppress insulin-like growth factor-1 (IGF-1) in bone tissue leading to deleterious effects on bone [170]. A human study showed that 14-week treatment with 8 mg/kg rosiglitazone significantly reduces bone formation markers like collagen type I and osteocalcin [171]. Both animal studies and clinical analysis have also reported that treatment with TZDs is associated with higher bone fracture risk [31,168,172].

Sulfonylureas are also widely used oral medications for T2DM that act by stimulating pancreatic β -cells to increase insulin secretion. The main defined sulfonylureas mechanism of action in the β -cells plasma membrane is by occupation of ATP-sensitive K-channels leading to insulin release [173]. Second generation sulfonylureas such as glimepiride and glibenclamide are more potent than the older ones [174]. To the best of our knowledge there is no report on beneficial effect of sulfonylureas on bone tissue except a report about positive indirect effect of sulfonylureas on bone tissue [135]. As we know this class of drugs act through insulin release and insulin is distinguished as a bone anabolic factor which acts through IRS signaling and glucose uptake regulation. Animal models indicated that in the lack of IRS genes, osteopenia and reduction in osteoblast/osteoclast function and impaired bone turnover would be more probable [175,176]. Insulinopenia in T1DM impairs osteoblast function and also bone formation markers like osteocalcin and procollagen type I will decrease due to insulinopenia [177]. Insulin therapy in diabetic patients can prevent osteopenia and osteoporosis [178].

Dipeptidyl peptidase 4 (DPP-4) inhibitors (like sitagliptin) are potent oral medications used for treatment of T2DM. The mechanism of action is based on stimulation of incretin release which results in decreasing glucagon levels, increasing insulin release, extending gastric emptying and reduction in glucose levels [179]. There are few studies demonstrating the positive effects of DPP-4 inhibitors on bone tissue. A study has demonstrated that DPP-4 inhibitors can decrease the risk of bone fractures in diabetic patients [180]. Another study has reported that DPP-4 inhibitors can protect osteoblasts from apoptosis and they have positive effects on bone tissue via collagen type 1 expression and alkaline phosphatase activity [181] as well as suppressing PTH-induced bone resorption [182]. In a recent study, Mamza and colleagues reported that there is no significant decrease in bone fracture after treatment with DPP-4 inhibitors [183].

As it has been mentioned above, aside from fewer side effects of metformin among all anti diabetic drugs, there is an important fact which gives metformin the capability of being used as an adjuvant therapy especially in bone disorders. It has been reported that metformin has no effect on glucose levels in non-diabetic individuals [62,63].

8. Conclusion

In this review authors discussed the association between diabetes and bone physiology, the link between AMPK, as the key molecule in metformin mechanism of action, and pathways involved in bone turnover, and the application of metformin as the most widely used oral antidiabetic drug in bone disorders and cancers. The association between metformin use and decreased risk of fractures has been reported in several clinical studies. Preclinical studies have also demonstrated the possible mechanisms involved in the positive effects of metformin on osteoblast differentiation and enhanced bone quality as a result. Since metformin has no substantial effect on glucose levels in non-diabetic individuals, it can be considered as a potential compound for adjuvant therapy of bone disorders in non-diabetic patients. In diabetics, further to positive osteogenic effects of metformin, glycemic control by metformin may help bone formation. Metformin has also showed beneficial effects on cancer treatment in various studies. Our studies have also showed that metformin could circumvent multidrug resistance in resistant breast cancer cells. Therefore, metformin can potentially reduce the migration of metastatic cancers into bone tissue. On the other hand, several studies have shown the anticancer effects of metformin in primary bone malignancies. Taken together, metformin can be considered as a potential compound for adjuvant therapy in bone disorders and malignancies further to its antidiabetic effects. However, more in vivo and clinical investigations are needed to concerning the safety and effective concentrations of metformin in bone diseases.

Conflicts of interest

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